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Die folgenden Angaben sind den vom Anmelder eingereichten Unterlagen entnommen

⑤④ Neuer metabotroper-Rezeptor-Komplex aus dem zentralen Nervensystem

⑤⑦ Die vorliegende Erfindung betrifft isolierte Proteine, die für neue metabotrope GABA-Rezeptoren kodieren und mit GABA<sub>B</sub>-Rezeptorproteinen einen neuen metabotroper GABA-Rezeptor-Komplex (= Proteinheteromer) bilden, und Nukleinsäuresequenzen oder rekombinante Nukleinsäurekonstrukte, die für die Proteine kodieren. Die Erfindung betrifft außerdem Wirtsorganismen oder transgene Tiere, enthaltend die Nukleinsäuresequenzen oder die rekombinanten Nukleinsäurekonstrukte sowie mono- oder polyklonale Antikörper, die gegen die isolierten Proteine gerichtet sind. Weiterhin betrifft die Erfindung ein Verfahren zum Auffinden von Substanzen mit spezifischer Bindungsaffinität an die erfindungsgemäßen neuen GABA-Rezeptoren bzw. die erfindungsgemäßen GABA-Rezeptor-Komplexe, ein Verfahren zum qualitativen oder quantitativen Nachweis der erfindungsgemäßen Nukleinsäuresequenzen oder der erfindungsgemäßen Proteine sowie einem Verfahren zum Auffinden von Substanzen, die spezifisch an einen erfindungsgemäßen GABA-Rezeptor oder an eine erfindungsgemäße Nukleinsäuresequenz binden. Ferner betrifft die Erfindung die Verwendung der Nukleinsäuresequenzen und Proteine.

GBR1A/B 591/475 FIG. 1  
SEQ2 481  
GBR1A/B 651/535 FIG. 2  
SEQ2 541  
GBR1A/B 711/595 FIG. 3  
SEQ2 598  
GBR1A/B 771/655 FIG. 4  
SEQ2 658  
GBR1A/B 811/715 FIG. 5  
SEQ2 718  
GBR1A/B 860/764 FIG. 6  
SEQ2 778  
GBR1A/B 911/795 FIG. 7  
SEQ2 833  
GBR1A/B 954/838 FIG. 8  
SEQ2 897

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Die vorliegende Erfindung betrifft isolierte Proteine, die für neue metabotrope GABA-Rezeptoren kodieren und mit GABA<sub>B</sub>-Rezeptorproteinen einen neuen metabotropen GABA-Rezeptor-Komplex (= Proteinheteromer) bilden, und Nukleinsäuresequenzen oder rekombinante Nukleinsäurekonstrukte, die für die Proteine kodieren.

Die Erfindung betrifft außerdem Wirtsorganismen oder transgene Tiere enthaltend die Nukleinsäuresequenzen oder die rekombinanten Nukleinsäurekonstrukte sowie mono- oder polyklonale Antikörper, die gegen die isolierten Proteine gerichtet sind.

Weiterhin betrifft die Erfindung ein Verfahren zum Auffinden von Substanzen mit spezifischer Bindungsaffinität an die erfindungsgemäßen neuen GABA-Rezeptoren bzw. die erfindungsgemäßen GABA-Rezeptor-Komplexe, ein Verfahren zum qualitativen oder quantitativen Nachweis der erfindungsgemäßen Nukleinsäuresequenzen oder der erfindungsgemäßen Proteine sowie einem Verfahren zum Auffinden von Substanzen, die spezifisch an einen erfindungsgemäßen GABA-Rezeptor oder an eine erfindungsgemäße Nukleinsäuresequenz binden. Ferner betrifft die Erfindung die Verwendung der Nukleinsäuresequenzen und Proteine.

Im zentralen Nervensystem von Vertebraten wird vornehmlich  $\gamma$ -Amino-Buttersäure (GABA) als inhibierender Neurotransmitter verwendet. GABA interagiert mit zwei Rezeptortypen GABA<sub>A</sub> und GABA<sub>B</sub>. Gut charakterisiert ist die Wirkung von GABA auf ionotrope Rezeptoren, die GABA<sub>A</sub>-Rezeptoren (Barnard et al., Trends Neurosci., 10, 1987: 502–509). Diese heteromeren Komplexe bilden Anionenkanäle, die auf Ligandenbindung hin geöffnet werden. Die Ausschüttung von GABA führt über die Aktivierung dieser Kanäle zu einem Chloridioneneinstrom, einem inhibitorischen postsynaptischen Strom, in die Zelle. GABA<sub>A</sub>-Rezeptoren sind die Angriffspunkte einer Reihe von Medikamenten wie beispielsweise von Benzodiazepinen, Barbituraten und anderen mehr [North (ed), 1994, Ligand- and Voltage Gated Ion Channels, in: Handbook of receptors and channels, vol. 2, CRC Press, Inc. and Smith and Olson, Trends Neurosci., 16, 1995: 162–168].

1981 wurden zum ersten Mal Bindungsstellen für GABA nachgewiesen, die unabhängig von Bindungsstellen auf GABA<sub>A</sub>-Rezeptoren vorliegen (Hill and Bowery, Nature, 290, 1981: 149–152). Sie befinden sich auf GABA-Rezeptoren, die intrazellulär an G-Proteine gekoppelt sind. Über diese G-Proteine sind diese Rezeptoren an neuronale Kalium- und Calciumkanäle gekoppelt. Diese neuen GABA-Rezeptoren (= GABA<sub>B</sub>-Rezeptoren) werden auch als metabotrope GABA-Rezeptoren bezeichnet. Diese GABA<sub>B</sub>-Rezeptoren sind über das zentrale und periphere Nervensystem verteilt (Ong et al., Life Sciences, Vol. 46, 1990: 1489–1501, Bowery et al., Drug Res., 42 (1), 1992: 215–223). Diese Rezeptoren werden sowohl prä- als auch postsynaptisch gefunden. An der Präsynapse kontrollieren die metabotropen GABA-Rezeptoren (= GABA<sub>B</sub>-Rezeptoren) die Ausschüttung verschiedener Neurotransmitter wie GABA, L-Glutamat, Noradrenalin, Dopamin, Serotonin, Substanz P, Cholecystokinin, Somatostatin und anderen. Liganden, Agonisten oder Antagonisten für GABA<sub>B</sub>-Rezeptoren, die die Ausschüttung eines spezifischen Neurotransmitters oder Neuropeptides regulieren, können benutzt werden, um Ungleichgewichte zwischen verschiedenen Neurotransmittersystemen, wie sie bei neurodegenerativen Erkrankungen, exzitotoxischen Begleiterscheinungen von neurologischen Erkrankungen sowie bei psychiatrischen Krankheiten vorkommen, auszugleichen. An der Postsynapse findet man GABA<sub>B</sub>-Rezeptoren, die über Gi-Proteine verschiedene Kalium-Kanäle aktivieren. Null-Mutationen für einen solchen Kanal in transgenen Mäusen führt zum Verlust der durch GABA bewirkten späten Inhibierung und dadurch zu spontanen Krämpfen. GABA<sub>B</sub>-Rezeptoren sind an Änderungen der synaptischen Effizienz beteiligt, die Lern- und Gedächtnisvorgängen zugrundeliegen. GABA<sub>B</sub>-Rezeptor-Agonisten zeigen positive Wirkung in Tiermodellen für chronische Schmerzen sowie Kokain-Abhängigkeit. Antagonisten wirken sich positiv in Modellen von "Absence-Epilepsie" aus (Bettler et al., Curr. Opin. Neurobiol., 8, 1998: 345–350). Aktivierung von GABA<sub>B</sub>-Rezeptoren dämpft übererregte neuronale Verknüpfungen. GABA<sub>B</sub>-Rezeptoren sind daher geeignete molekulare Targets für die Behandlung von Epilepsie, Schlaganfall, kognitiven Verlusten, chronischen Schmerzen und weiteren neurologischen Erkrankungen sowie für die Behandlung von psychischen Erkrankungen wie Angst, depressiven Erkrankungen, Schizophrenie, Migräne und anderen. Sie sind auch als Targets für die Therapie von Kokain-Abhängigen sowie als Angriffspunkt für neuartige kognitive Enhancer geeignet. Ein GABA<sub>B</sub>-Rezeptor-Agonist, Baclofen (Lioresal), wird für die Behandlung von multipler Sklerose und bei den Folgen von Rückenmarksverletzungen klinisch eingesetzt (Bowery, Annu. Rev. Pharmacol. Toxicol., 33, 1993: 109–147).

Auch im peripheren Nervensystem (PNS) werden positive Effekte von GABA<sub>B</sub>-Rezeptor-Agonisten vermutet, beispielsweise bei Entzündungen und Atemwegserkrankungen.

Einzelne biochemische und pharmakologische Erkenntnisse können möglicherweise dahingehend interpretiert werden, daß im zentralen Nervensystem mehrere GABA<sub>B</sub>-Rezeptor-Subtypen mit verschiedenen Funktionen existieren. Bisher wurde die cDNA für einen GABA<sub>B</sub>-Rezeptor gefunden, der in zwei aminoterminalen Spleissformen auftritt, welche sich pharmakologisch kaum unterscheiden (Kaupmann et al., Nature, 386, 239–246, 1997, WO97/46675). Dieser GABA<sub>B</sub>-Rezeptor weist nach Expression in heterologen Systemen ähnlich hohe Affinitäten zu den bekannten GABA<sub>B</sub>-Rezeptor-Antagonisten auf, wie sie im Gehirn gefunden werden. Die Affinitäten von GABA<sub>B</sub>-Rezeptor-Agonisten zu diesem klonierten GABA<sub>B</sub>-Rezeptor liegen dagegen etwa um den Faktor 100 unter den im Gehirn gemessenen Werten (Kaupmann et al., Nature, 386, 239–246, 1997). Verschiedene GABA<sub>B</sub>-Rezeptorantagonisten und -agonisten und ihre Wirkung sind Froestl et al., J. Med. Chem. Vol. 38, 1995: 3297–3312 und 3313–3331 zu entnehmen.

Da GABA<sub>B</sub>-Rezeptoren eine zentrale Rolle bei verschiedenen pathologischen Prozessen des zentralen und peripheren Nervensystems spielen bzw. an derartigen Prozessen beteiligt sind, sind sie gesuchte Targets für die Entwicklung neuer Pharmaka.

Es bestand daher die Aufgabe neue GABA<sub>B</sub>-Rezeptoren oder Proteine, die mit den GABA<sub>B</sub>-Rezeptoren interagieren, zu identifizieren und zu charakterisieren, die möglichst hochaffine Bindungsstellen für GABA<sub>B</sub>-Rezeptor-Agonisten und -Antagonisten besitzen und die damit die Entwicklung molekularer Testsysteme ermöglichen, mit denen man viele Tausend verschiedene Verbindungen nach hochaffinen Substanzen in kurzer Zeit durchsuchen kann. Diese so charakterisierten und spezifisch am GABA<sub>B</sub>-Rezeptor angreifenden Substanzen sind potentielle Kandidaten für Wirkstoffe gegen Krankheiten wie Epilepsie, Schlaganfall, psychische Erkrankungen wie Angst, manisch-depressive Erkrankungen, Schi-

zophrenie, Migräne und andere mehr.

Diese Aufgabe wurde mit dem erfindungsgemäßen Proteinheteromer, enthaltend mindestens ein GABA<sub>B</sub>-Rezeptorprotein und mindestens ein Protein mit der in SEQ ID NO: 2 oder SEQ ID NO: 4 dargestellte Aminosäuresequenz oder eine daraus durch Substitution, Inversion, Insertion oder Deletion von einem oder mehreren Aminosäureresten erhaltliche Sequenz, wobei wenigstens noch eine der wesentlichen biologischen Eigenschaften des in SEQ ID NO: 2 oder SEQ ID NO: 4 dargestellten Proteins oder des Proteinheteromer erhalten bleibt bzw. mit dem isolierten Protein, enthaltend die in SEQ ID NO: 2 oder SEQ ID NO: 4 dargestellte Aminosäuresequenz oder eine daraus durch Substitution, Inversion, Insertion oder Deletion von einem oder mehreren Aminosäureresten erhaltliche Sequenz, wobei wenigstens noch eine der wesentlichen biologischen Eigenschaften des in SEQ ID NO: 2 oder SEQ ID NO: 4 dargestellten Proteins erhalten bleibt, gelöst.

Unter den erfindungsgemäßen Proteinheteromeren sind GABA-Rezeptor-Komplexe vorteilhaft metabotrope GABA-Rezeptor-Komplexe enthaltend mindestens einen GABA<sub>B</sub>-Rezeptorprotein und mindestens ein Protein mit der in SEQ ID NO: 2 oder SEQ ID NO: 4 dargestellten Aminosäuresequenz zu verstehen. In WO97/46675 werden geeignete GABA<sub>B</sub>-Rezeptorproteine, die vorteilhaft in den Proteinheteromeren enthalten sein können, beschrieben. Diese GABA<sub>B</sub>-Rezeptorproteine tragen in WO97/46675 die Sequenzbezeichnungen bzw. Klonnamen "SEQ ID NO: 1" bzw. "GABABR1a rat" (kloniert aus *Rattus norvegicus*), "SEQ ID NO: 3" bzw. "GABABR1a/b human" (kloniert aus *Homo sapiens*), "SEQ ID NO: 5" bzw. "GABABR1b rat" (kloniert aus *Rattus norvegicus*) und "SEQ ID NO: 7" bzw. "GABABR1b human" (kloniert aus *Homo sapiens*). Auf diese Rezeptoren und die Schrift WO97/46675 wird an dieser Stelle ausdrücklich bezug genommen.

Unter den erfindungsgemäßen isolierten Proteinen sind Proteine zu verstehen, die eine in SEQ ID NO: 2 oder SEQ ID NO: 4 dargestellte Aminosäuresequenz oder eine daraus durch Substitution, Inversion, Insertion oder Deletion von einem oder mehreren Aminosäureresten erhaltliche Sequenz enthalten, wobei wenigstens noch eine der wesentlichen biologischen Eigenschaften des in SEQ ID NO: 2 oder SEQ ID NO: 4 dargestellten Proteins erhalten bleibt. Dabei können beispielsweise bestimmte Aminosäuren durch solche mit ähnlichen physikochemischen Eigenschaften (Raumerfüllung, Basizität, Hydrophobizität etc.) ersetzt werden. Beispielsweise werden Argininreste gegen Lysinreste, Valinreste gegen Isoleucinreste oder Asparaginsäurereste gegen Glutaminsäurereste ausgetauscht. Es können aber auch ein oder mehrere Aminosäuren in ihrer Reihenfolge vertauscht, hinzugefügt oder entfernt werden, oder es können mehrere dieser Maßnahmen miteinander kombiniert werden. Die solchermaßen gegenüber der SEQ ID NO: 2 oder SEQ ID NO: 4 veränderten Proteine besitzen wenigstens 60%, bevorzugt wenigstens 70% und besonders bevorzugt wenigstens 90% Sequenzidentität zu den Sequenzen SEQ ID NO: 2 oder SEQ ID NO: 4 berechnet nach dem Algorithmus von "Altschul et al., J. Mol. Biol., 215, 403-410, 1990".

Unter der wesentlichen biologischen Eigenschaft der erfindungsgemäßen Proteine bzw. Proteinheteromere sind der oder die transmembranen Bereiche, der aminoterminal Bereich und wesentlich der carboxyterminale Bereich des Proteins allein oder im Proteinheteromer zu verstehen (siehe Fig. 2). Diese Proteinbereiche ermöglichen die spezielle biologische Wirkung der Proteine bzw. Proteinheteromere. Diese wesentlichen biologischen Eigenschaften beinhalten außerdem die hochaffine Bindung ( $K_d < 10 \text{ nM}$ ) spezifischer synthetischer oder natürlicher Agonisten und Antagonisten an der erfindungsgemäßen Proteine mit der in SEQ ID NO: 2 oder SEQ ID NO: 4 dargestellte Aminosäuresequenz, die Signalweiterleitung an ein intrazelluläres G-Protein sowie die Interaktion mit den oben genannten, bekannten GABA<sub>B</sub>-Rezeptoren.

Das isolierte Protein und seine funktionellen Varianten lassen sich vorteilhafterweise aus dem Gehirn von Mammalia wie *Homo sapiens* oder *Rattus norvegicus* isolieren. Auch Homologe aus anderen Mammalia sind unter funktionellen Varianten zu verstehen.

Ein weiterer Gegenstand der Erfindung sind Nukleinsäuresequenzen, die für die oben beschriebenen Proteine kodieren, insbesondere für solche mit der in SEQ ID NO: 2 oder SEQ ID NO: 4 dargestellten Primärstruktur. Die Nukleinsäuresequenz aus *Rattus norvegicus* oder *Homo sapiens* ist in SEQ ID NO: 1 bzw. in SEQ ID NO: 3 dargestellt.

Nach Isolierung und Sequenzierung sind die erfindungsgemäßen Nukleotidsequenzen SEQ ID NO: 1 und SEQ ID NO: 3 oder deren funktionelle Äquivalente wie z. B. Allelvarianten erhältlich. Unter Allelvarianten sind SEQ ID NO: 1- oder SEQ ID NO: 3-Varianten zu verstehen, die 60 bis 100% Homologie auf Aminosäureebene, bevorzugt 70 bis 100%, ganz besonders bevorzugt 90 bis 100% aufweisen. Allelvarianten umfassen insbesondere solche funktionellen Varianten, die durch Deletion, Insertion oder Substitution von Nukleotiden aus der in SEQ ID NO: 1 oder SEQ ID NO: 3 dargestellten Sequenz erhältlich sind, wobei wenigstens noch eine der wesentlichen biologischen Eigenschaften erhalten bleibt. Homologe oder sequenzverwandte Nukleinsäuresequenzen können aus allen Säugerspezies einschließlich Mensch nach gängigen Verfahren durch Homologiescreening durch Hybridisierung mit einer Probe der erfindungsgemäßen Nukleinsäuresequenzen oder Teilen davon isoliert werden.

Unter funktionellen Äquivalenten sind auch Homologe von SEQ ID NO: 1 oder SEQ ID NO: 3 beispielsweise ihre Homologen aus anderen Mammalia, verkürzte Sequenzen, Einzelstrang-DNA oder RNA der codierenden und nichtcodierenden DNA-Sequenz zu verstehen.

Solche funktionellen Äquivalente lassen sich ausgehend von den in SEQ ID NO: 1 oder SEQ ID NO: 3 beschriebenen DNA-Sequenzen oder Teilen dieser Sequenzen, beispielsweise mit üblichen Hybridisierungsverfahren oder der PCR-Technik aus anderen Vertebraten wie Mammalia isolieren. Diese DNA-Sequenzen hybridisieren unter Standardbedingungen mit den erfindungsgemäßen Sequenzen. Zur Hybridisierung werden vorteilhaft kurze Oligonukleotide der konservierten Bereiche beispielsweise aus den Transmembranbereichen oder aus dem aminoterminalen Bereich, die über Vergleiche mit anderen Transmembranproteinen speziell anderen GABA-Rezeptoren in dem Fachmann bekannter Weise ermittelt werden können, verwendet. Es können aber auch längere Fragmente der erfindungsgemäßen Nukleinsäuren oder die vollständigen Sequenzen für die Hybridisierung verwendet werden. Je nach der verwendeten Nukleinsäure Oligonukleotid, längeres Fragment oder vollständige Sequenz oder je nachdem welche Nukleinsäureart DNA oder RNA für die Hybridisierung verwendet werden, variieren diese Standardbedingungen. So liegen beispielsweise die Schmelztemperaturen für DNA : DNA-Hybride ca 10°C niedriger als die von DNA : RNA-Hybriden gleicher Länge.

Unter Standardbedingungen sind beispielsweise je nach Nukleinsäure Temperaturen zwischen 42 und 58°C in einer wäßrigen Pufferlösung mit einer Konzentration zwischen 0,1 bis 5 × SSC (1 × SSC = 0,15 M NaCl, 15 mM Natriumcitrat, pH 7,2) oder zusätzlich in Gegenwart von 50% Formamid wie beispielsweise 42°C in 5 × SSC, 50% Formamid zu verstehen. Vorteilhafterweise liegen die Hybridisierungsbedingungen für DNA : DNA-Hybride bei 0,1 × SSC und Temperaturen zwischen etwa 20°C bis 45°C, bevorzugt zwischen etwa 30°C bis 45°C. Für DNA : RNA-Hybride liegen die Hybridisierungsbedingungen vorteilhaft bei 0,1 × SSC und Temperaturen zwischen etwa 30°C bis 55°C, bevorzugt zwischen etwa 45°C bis 55°C. Diese angegebenen Temperaturen für die Hybridisierung sind beispielhaft kalkulierte Schmelztemperaturwerte für eine Nukleinsäure mit einer Länge von ca. 100 Nukleotiden und einem G + C-Gehalt von 50% in Abwesenheit von Formamid. Die experimentellen Bedingungen für die DNA-Hybridisierung sind in einschlägigen Lehrbüchern der Genetik wie beispielsweise Sambrook et al., "Molecular Cloning", Cold Spring Harbor Laboratory, 1989, beschrieben und lassen sich nach dem Fachmann bekannten Formeln beispielsweise abhängig von der Länge der Nukleinsäuren, der Art der Hybride oder dem G + C-Gehalt berechnen. Weitere Informationen zur Hybridisierung kann der Fachmann folgenden Lehrbüchern entnehmen: Ausubel et al. (eds), 1998, Current Protocols in Molecular Biology, John Wiley & Sons, New York; Hames and Higgins (eds), 1985, Nucleic Acids Hybridization: A Practical Approach, IRL Press at Oxford University Press, Oxford; Brown (ed), 1991, Essential Molecular Biology: A Practical Approach, IRL Press at Oxford University Press, Oxford.

Außerdem sind unter Homologe der Sequenzen SEQ ID NO: 1 und SEQ ID NO: 3 Derivate wie beispielsweise Promotorvarianten zu verstehen. Die Promotoren, die den angegebenen Nukleotidsequenzen gemeinsam oder einzeln vorgeschaltet sind, können durch ein oder mehrere Nukleotidaustausche, durch Insertion(en) und/oder Deletion(en) verändert sein, ohne daß aber die Funktionalität bzw. Wirksamkeit der Promotoren beeinträchtigt sind. Des weiteren können die Promotoren durch Veränderung ihrer Sequenz in ihrer Wirksamkeit erhöht oder komplett durch wirksamere Promotoren auch artfremder Organismen ausgetauscht werden.

Unter Derivaten sind auch vorteilhaft Varianten zu verstehen, deren Nukleotidsequenz im Bereich -1 bis -1000 vor dem Startkodon so verändert wurden, daß die Genexpression und/oder die Proteinexpression verändert, bevorzugt erhöht wird. Weiterhin sind unter Derivaten auch Varianten zu verstehen, die am 3'-Ende verändert wurden.

Für eine optimale Expression heterologer Gene in Organismen ist es vorteilhaft die Nukleinsäuresequenzen entsprechend des im Organismus verwendeten spezifischen "codon usage" zu verändern. Der "codon usage" läßt sich anhand von Computerauswertungen anderer, bekannter Gene des betreffenden Organismus leicht ermitteln.

Weiterhin ist es von Vorteil die erfindungsgemäßen Nukleinsäuren SEQ ID NO: 1 oder SEQ ID NO: 3 allein oder die Nukleinsäuren SEQ ID NO: 1 oder SEQ ID NO: 3 und eine Sequenz, die für einen GABA<sub>A</sub>-Rezeptorprotein kodiert, funktionell mit mindestens einem genetischen Regulationselement zu den erfindungsgemäßen rekombinanten Nukleinsäurekonstrukten zu verknüpfen.

Dazu werden die erfindungsgemäßen Nukleinsäuresequenzen üblicherweise mit genetischen Regulationselementen wie Transkriptions- und Translationssignalen funktionell verknüpft. Diese Verknüpfung kann je nach gewünschter Anwendung zu einer Erhöhung oder Erniedrigung der Genexpression führen. Mit den solchermaßen hergestellten rekombinanten Nukleinsäurekonstrukten werden anschließend Wirtsorganismen transformiert. Zusätzlich zu diesen neuen Regulationssequenzen kann die natürliche Regulation dieser Sequenzen vor den eigentlichen Strukturgenen noch vorhanden sein und gegebenenfalls genetisch verändert worden sein, so daß die natürliche Regulation ausgeschaltet und die Expression der Gene erhöht wurde. Das Genkonstrukt kann aber auch einfacher aufgebaut sein, das heißt es werden keine zusätzlichen Regulationssignale vor die Sequenzen inseriert und der natürliche Promotor mit seiner Regulation wird nicht entfernt. Stattdessen wird die natürliche Regulationssequenz so mutiert, daß keine Regulation mehr erfolgt und die Genexpression gesteigert wird. Auch am 3'-Ende der Nukleinsäure-Sequenzen können zusätzliche vorteilhafte regulatorische Elemente inseriert werden. Die Nukleinsäuresequenzen für die Sequenzen SEQ ID NO: 1 oder SEQ ID NO: 3 und/oder für die GABA<sub>A</sub>-Rezeptorproteine können in einer oder mehreren Kopien im Genkonstrukt enthalten sein, oder auf getrennten Genkonstrukten lokalisiert sein.

Vorteilhafte Regulationssequenzen für das erfindungsgemäße Verfahren sind beispielsweise in Promotoren wie cos-, lac-, trp-, tet-, trp-tet-, lpp-, lac-, lpp-lac-, lacIq-, T7-, T5-, T3-, gal-, trc-, ara-, SP6-, 1-PR- oder im 1-PI-Promotor enthalten, die vorteilhafterweise in gram-negativen Bakterien Anwendung finden. Weitere vorteilhafte Regulationssequenzen sind beispielsweise in den gram-positiven Promotoren wie amy und SPO2, in den Hefepromotoren wie ADC1, MFa, AC, P-60, CYC1, GAPDH oder in Mammaliapromotoren wie CaM-KinaseII, CMV, Nestin, L7, BDNF, NF, MBP, NSE, β-Globin, GFAP, GAP43, Tyrosin Hydroxylase, Kainat-Rezeptor-Untereinheit 1, Glutamat-Rezeptor-Untereinheit B enthalten.

Prinzipiell können alle natürlichen Promotoren mit ihren Regulationssequenzen wie die oben genannten verwendet werden. Darüberhinaus können auch synthetische Promotoren vorteilhaft verwendet werden.

Diese regulatorischen Sequenzen sollen die gezielte Expression der Nukleinsäuresequenzen und der Proteinexpression ermöglichen. Dies kann beispielsweise je nach Wirtsorganismus bedeuten, daß das Gen erst nach Induktion exprimiert oder überexprimiert wird, oder daß es sofort exprimiert und/oder überexprimiert wird.

Die regulatorischen Sequenzen bzw. Faktoren können dabei vorzugsweise die Expression positiv beeinflussen und dadurch erhöhen. So kann eine Verstärkung der regulatorischen Elemente vorteilhafterweise auf der Transkriptionsebene erfolgen, indem starke Transkriptionssignale wie Promotoren und/oder "Enhancer" verwendet werden. Daneben ist aber auch eine Verstärkung der Translation möglich, indem beispielsweise die Stabilität der mRNA verbessert wird.

Unter "Enhancer" sind beispielsweise DNA-Sequenzen zu verstehen, die über eine verbesserte Wechselwirkung zwischen RNA-Polymerase und DNA eine erhöhte Expression bewirken. Als weitere Regulationssequenzen seien beispielhaft die locus control regions, silencer oder jeweilige Teilsequenzen davon genannt. Diese Sequenzen können vorteilhaft für eine gewebespezifische Expression verwendet werden.

Eine bevorzugte Ausführungsform ist die Verknüpfung der erfindungsgemäßen Nukleinsäuresequenz mit einem Promotor, wobei der Promotor 5' up stream zu liegen kommt. Weitere Regulationssignale wie 3' gelegene Terminatoren oder Polyadenylierungssignale oder Enhancer können funktionell in dem Nukleinsäurekonstrukt Anwendung finden.



Unter dem Begriff des erfindungsgemäßen "rekombinanten Nukleinsäurekonstrukts bzw. Genkonstrukts" sind auch komplette Vektorkonstrukte zu verstehen. Diese Vektorkonstrukte oder Vektoren werden zur Expression in einem geeigneten Wirtsorganismus verwendet. Vorteilhafterweise werden die erfindungsgemäßen Nukleinsäuren und/oder die Gene für die GABA<sub>B</sub>-Rezeptoren in einen wirtsspezifischen Vektor inseriert, der eine optimale Expression der Gene im ausgesuchten Wirt ermöglicht. Vektoren sind dem Fachmann wohl bekannt und können beispielsweise aus dem Buch Cloning Vectors (Eds. Pouwels P. H. et al. Elsevier, Amsterdam-New York-Oxford, 1985, ISBN 0 444 904018) entnommen werden. Unter Vektoren sind außer Plasmiden auch alle anderen dem Fachmann bekannten Vektoren wie beispielsweise Phagen, Viren wie SV40, CMV, Baculovirus, Adenovirus, Transposons, IS-Elemente, Phasmide, Phagemide, Cosmide, lineare oder zirkuläre DNA zu verstehen. Diese Vektoren können autonom im Wirtsorganismus repliziert oder chromosomal repliziert werden. Für die Integration in Mammalia wird vorteilhaft lineare DNA verwendet.

Die Expression der erfindungsgemäßen Nukleinsäuresequenzen bzw. des rekombinanten Nukleinsäurekonstrukts kann vorteilhaft durch Erhöhen der Genkopienzahl und/oder durch Verstärkung regulatorischer Faktoren, die die Genexpression positiv beeinflussen, erhöht werden. So kann eine Verstärkung regulatorischer Elemente vorzugsweise auf der Transkriptionsebene erfolgen, indem stärkere Transkriptionssignale wie Promotoren und Enhancer verwendet werden. Daneben ist aber auch eine Verstärkung der Translation möglich, indem beispielsweise die Stabilität der mRNA verbessert, oder die Ableseseffizienz dieser mRNA an den Ribosomen erhöht wird.

Zur Erhöhung der Genkopienzahl können die Nukleinsäuresequenzen oder homologe Gene, beispielsweise in ein Nukleinsäurefragment bzw. in einen Vektor eingebaut werden, der vorzugsweise die den jeweiligen Genen zugeordnete, regulatorische Gensequenzen oder analog wirkende Promotoraktivität enthält. Insbesondere werden solche regulatorische Sequenzen verwendet, die die Genexpression verstärken.

Die erfindungsgemäßen Nukleinsäuresequenzen können zusammen mit den für die GABA<sub>B</sub>-Rezeptoren kodierenden Sequenzen in einen einzelnen Vektor kloniert werden und anschließend in dem gewünschten Organismus exprimiert werden. Alternativ kann auch jede der beschriebenen Nukleinsäuresequenzen und die für die GABA<sub>B</sub>-Rezeptoren kodierenden Sequenzen in je einen einzelnen Vektor gebracht und diese getrennt in den jeweiligen Organismus über übliche Methoden wie Transformation, Transfektion, Transduktion, Elektrophoration oder Partikel-Gun verbracht werden.

Darüberhinaus kann das erfindungsgemäße Nukleinsäurekonstrukt oder die erfindungsgemäßen Nukleinsäuren auch in Form therapeutisch oder diagnostisch geeigneter Fragmente exprimiert werden. Zur Generierung des rekombinanten Proteins können Vektorsysteme oder Oligonukleotide verwendet werden, die die Nukleinsäuren oder das Nukleinsäurekonstrukt um bestimmte Nukleotidsequenzen verlängern und damit für veränderte Polypeptide kodieren, die einer einfacheren Reinigung dienen. Als solche "Tags" sind in der Literatur z. B. Hexa-Histidin-Anker bekannt oder Epitope, die als Antigene verschiedener Antikörper erkannt werden können (Studier et al., Meth. Enzymol., 185, 1990: 60-89 und Ausubel et al. [eds.]m 1998, Current Protocols in Molecular Biology, John Wiley & Sons, New York).

Als Wirtsorganismen sind prinzipiell alle Organismen geeignet, die eine Expression der erfindungsgemäßen Nukleinsäuren, ihrer Allelvarianten, ihrer funktionellen Äquivalente oder Derivate oder des rekombinanten Nukleinsäurekonstrukts allein oder zusammen mit einer Sequenz, die für GABA<sub>B</sub>-Rezeptorproteine kodiert, ermöglichen. Unter Wirtsorganismen sind beispielsweise Bakterien, Pilze, Hefen, pflanzliche oder tierische Zellen zu verstehen. Bevorzugte Organismen sind Bakterien wie *Escherichia coli*, *Streptomyces*, *Bacillus* oder *Pseudomonas*, eukaryotische Mikroorganismen wie *Saccharomyces cerevisiae*, *Aspergillus*, höhere eukaryotische Zellen aus Mensch oder Tier, beispielsweise COS-, Hela-, HEK293-, Sf9- oder CHO-Zellen.

Gewünschtenfalls kann das Genprodukt auch in transgenen Organismen wie transgenen Tieren z. B. Mäusen, Ratten, Schafen, Rindern oder Schweinen zur Expression gebracht werden. Auch transgene Pflanzen sind im Prinzip denkbar. Bei den transgenen Organismen kann es sich auch um sogenannte Knock-Out Tiere handeln.

Dabei können die transgenen Tiere eine funktionelle oder nicht funktionelle erfindungsgemäße Nukleinsäuresequenz oder ein funktionelles oder nicht funktionelles Nukleinsäurekonstrukt allein oder in Kombination mit einer funktionellen oder nicht funktionellen Sequenz, die für GABA<sub>B</sub>-Rezeptorproteine kodiert, enthalten.

Eine weitere erfindungsgemäße Ausgestaltung der oben beschriebenen transgenen Tiere sind transgene Tiere, in dessen Keimzellen oder der Gesamtheit oder einem Teil der somatischen Zellen; oder in dessen Keimzellen und der Gesamtheit oder einem Teil der somatischen Zellen die erfindungsgemäßen Nukleotidsequenz durch gentechnische Verfahren verändert oder durch Einfügen von DNA-Elementen unterbrochen wurden.

Die Kombination aus dem Wirtsorganismen und den zu den Organismen passenden Vektoren wie Plasmide, Viren oder Phagen wie beispielsweise Plasmide mit dem RNA-Polymerase/Promoter System, die Phagen 1, Mu oder andere temperante Phagen oder Transposons und/oder weiteren vorteilhaften regulatorischen Sequenzen bilden ein Expressionssystem. Bevorzugt sind unter dem Begriff Expressionssysteme beispielsweise die Kombination aus Säugetierzellen wie CHO-Zellen und vektoren wie pcDNA3neo-Vektor oder HEK293-Zellen und CMV-Vektor, die für Säugetierzellen geeignet sind, zu verstehen.

Die In-situ Hybridisierung mit der Sequenz SEQ ID NO: 2 oder Teilen davon ergab eine starke Expression in Hippokampus, Cortex, Cerebellum sowie in thalamischen Kernen (siehe Fig. 1 und Beispiele). Fig. 1a und 1b geben die Expressionsanalyse der zu SEQ ID NO: 1 korrespondierenden mRNA wieder. 1a zeigt den Northern blot, 1b die in situ-Hybridisierung (siehe Beispiel 3 und 4). Das Expressionsmuster überlappt mit dem des GABA<sub>B</sub>-Rezeptors und deutet auf eine wichtige zentralnervöse Funktion des in SEQ ID NO: 2 oder SEQ ID NO: 4 dargestellten Proteins hin. Der Hippokampus ist die entscheidende Hirnstruktur für die Speicherung neuer Gedächtnisinhalte. Ein Protein mit der Sequenz SEQ ID NO: 2 ist damit ein interessantes Target für das Verständnis in Bezug auf Lernen und Gedächtnis und für die Entwicklung neuer kognitiver Enhancer. Als Teil des limbischen Systems beeinflusst der Hippocampus auch Stimmungen und Gefühle. Gegen SEQ ID NO: 2 oder SEQ ID NO: 4 und ihre funktionellen Äquivalente, Homologe oder Derivate gerichtete Pharmaka stellen damit potentielle Antidepressiva oder Anxiolytika dar und können bei kognitiven Erkrankungen verwendet werden. Schließlich ist der Hippocampus stark in Temporallappenepilepsien involviert, was ein Protein mit SEQ ID NO: 2 oder SEQ ID NO: 4 zu einem attraktiven Target für neue Medikamente gegen diese häufige Erkrankung macht. Im Cortex befinden sich Regionen, die sensorische Informationen integrieren, verarbeiten und in geeignete

Reaktionen umwandeln. Diese sensorischen und motorischen Zentren sind oft auch Ausgangspunkte für epileptische Anfälle. Eine gezielte Beeinflussung der erfindungsgemäßen Proteine oder des erfindungsgemäßen Proteinheteromers könnte die Krampfwahrscheinlichkeit bei Epilepsiepatienten senken. Die thalamischen Kerne sind dem Cortex vorgeschaltet, integrieren die über die Sinnesorgane aufgenommenen Wahrnehmungen und leiten sie an corticale Strukturen weiter. Sie sind häufig der Ausgangspunkt für generalisierte Krampfanfälle. Die starke Expression der erfindungsgemäßen Proteine oder des Proteinheteromers in den thalamischen Kernen deutet darauf hin, daß seine Aktivierung oder seine Inhibition zur Linderung von Krampfanfällen in Epilepsiepatienten beitragen kann. Die cerebellären Verknüpfungen sind maßgeblich für die Feinkoordination der Bewegungen verantwortlich. Ataxien und andere motorische Erkrankungen könnten auf einer Deregulation eines Proteins mit der erfindungsgemäßen Nukleinsäuresequenz beruhen. Die erfindungsgemäßen Proteinheteromere oder Proteine stellen damit interessante Targets für die Entwicklung neuer Substanzen dar, die zur Herstellung von Medikamenten zur Behandlung von Krankheiten wie neurologische Erkrankungen wie Epilepsie, Schlaganfall, psychische Erkrankungen wie Angst, manisch-depressive Erkrankungen, Migräne, kognitive Verluste und weiteren neurologischen Erkrankungen.

Substanzen die eine Wirkung gegenüber GABA<sub>B</sub>-Rezeptoren aufweisen sind beispielsweise als Agonist Baclofen und seine Derivate und als Antagonist Phaclofen, Saclofen und Derivate. Diese Substanzen sowie weitere wirksame Substanzen sind J. Med. Chem., 38, 1995: 3313-3331, J. Med. Chem., 38, 1995: 3297-3312 und WO97/46675 zu entnehmen. Diese Substanzen wirken mit hoher Wahrscheinlichkeit auch gegenüber dem erfindungsgemäßen Proteinheteromer oder dem erfindungsgemäßen Protein. Vermutlich lassen sich mit Hilfe der erfindungsgemäßen Proteine (= Proteinheteromer + isoliertes Protein) leichter agonistisch stärker wirkende Substanzen entwickeln. Mit Hilfe der neuen Proteine können selektivere Substanzen entwickelt werden.

Das Gen für den bereits bekannten GABA<sub>B</sub>-Rezeptor befindet sich in der Nähe des chromosomalen Locus, der mit der juvenilen, myoclonischen Epilepsie assoziiert ist. Diese Korrelation könnte außerdem ein neues Diagnoseverfahren für diese verbreitete Epilepsieform ermöglichen. Gleiches gilt für die erfindungsgemäßen Proteine. Die Nukleotidsequenzen SEQ ID NO: 1 und SEQ ID NO: 3 kann dazu verwendet werden, Gene für mRNAs, die für diese Nukleinsäuren oder deren funktionellen Äquivalente, Homologe oder Derivate kodieren, im murinen und im menschlichen Genom mit gängigen Methoden durch Homologiescreening zu isolieren und zu kartieren und mit Markern für humane Erbkrankheiten zu korrelieren. Dies ermöglicht die Identifizierung des Gens als Ursache für bestimmte Erbkrankheiten, was ihre Diagnose erheblich vereinfacht und neue Therapieansätze ermöglicht. Mit Hilfe der Nukleinsäuren als Marker lassen sich damit Erbkrankheiten diagnostizieren.

Die Erfindung betrifft außerdem die Verwendung der erfindungsgemäßen Nukleinsäuren oder Teilen davon zur Gentherapie. Auch zu den erfindungsgemäßen Nukleinsäure oder Teilen davon komplementäre Sequenzen können zur Gentherapie verwendet werden.

Eine weitere Möglichkeit des Einsatzes der Nukleotidsequenz oder Teilen davon ist die Erzeugung transgener oder knock-out- oder konditioneller oder regionenspezifischer knock-out Tiere oder spezifischer Mutationen in gentechnisch veränderten Tieren (Ausubel et al. [eds]. 1998, Current Protocols in Molecular Biology, John Wiley & Sons, New York und Torres et al., [eds.] in 1997, Laboratory protocols for conditional gene targeting, Oxford University Press, Oxford). Über transgene überexpression oder genetische Mutation (Nullmutation oder spezifische Deletionen, Insertionen oder Veränderungen) durch homologe Rekombination in embryonalen Stammzellen kann man Tiermodelle erzeugen, die wertvolle weitere Informationen über die (Patho-)Physiologie der erfindungsgemäßen Sequenzen allein oder in Komplex mit dem GABA<sub>B</sub>-Rezeptor liefern. Solchermaßen hergestellte Tiermodelle können essentielle Testsysteme zur Evaluierung neuartiger Therapeutika darstellen, die die Signaltransduktion von GABA<sub>B</sub>-Rezeptoren beeinflussen.

Die Interaktion des bekannten GABA<sub>B</sub>-Rezeptors mit dem neuen erfindungsgemäßen, beschriebenen 7-Transmembranregionen-Protein, das mit dem two-hybrid System entdeckt wurde, spielt eine wichtige physiologische Rolle. Dieser überraschende Befund ermöglicht neue außerordentliche Behandlungsmöglichkeiten im Hinblick auf die o. g. neurologischen und psychischen Erkrankungen, die mit dem GABA<sub>B</sub>-Rezeptor im Zusammenhang stehen. Niedermolekulare Effektoren oder Peptide, die diese Interaktion positiv oder negativ beeinflussen, sind Wirkstoffe, die in die GABAerge Signaltransduktion eingreifen und damit als neue Klasse von Pharmaka zum Einsatz kommen können. Die direkte molekulare Interaktion zwischen zwei unterschiedlichen metabotropen Rezeptoren war bislang nicht bekannt. Ebenso sind bislang keine Substanzen beschrieben, die die Interaktion zwischen zwei unterschiedlichen metabotropen Rezeptoren beeinflussen und dadurch die Signaltransduktion über diese Rezeptoren modulieren. Die Verwendung des erfindungsgemäßen Proteinheteromers ermöglicht damit die Entwicklung neuer Wirkstoffe bzw. Wirkstoffklassen.

Durch Verwendung der erfindungsgemäßen Nukleinsäuresequenz, des Nukleinsäurekonstrukts, eines erfindungsgemäßen Proteinheteromeren oder des Proteins können Proteine identifiziert werden, die zum Proteinheteromeren oder zum Protein spezifische Bindungsaffinitäten aufweisen, oder zur Identifizierung von Nukleinsäuren, die für Proteine kodieren, die zum Proteinheteromeren oder zum Protein spezifische Bindungsaffinitäten aufweisen. Vorteilhafterweise werden hierzu das two-hybrid System oder andere biochemische Verfahren allein oder in Kombination verwendet. Es lassen sich so Interaktionsdomänen von metabotropen Rezeptoren und damit pharmakotherapeutische Interventionspunkte bestimmen.

Daher ist ein Gegenstand der Erfindung die Verwendung des two-hybrid Systems oder biochemischer Verfahren zur Identifizierung der Interaktionsdomänen von metabotropen Rezeptoren und die Verwendung zur pharmakotherapeutischen Intervention.

Über Strukturanalysen des Proteinheteromers oder des erfindungsgemäßen Proteins lassen sich gezielt Substanzen finden, die eine spezifische Bindungsaffinität aufweisen.

Die beschriebenen Sequenzen SEQ ID NO: 1 und SEQ ID NO: 3 ermöglichen, mit Hilfe des two-hybrid Systems oder anderen Assays, die für die Interaktion verantwortlichen Aminosäuren einzugrenzen und Substanzen zu finden, mit denen die Interaktion zwischen den beiden metabotropen Rezeptoren beeinflusst werden kann. Die Verwendung von Substanzen, die die physikalische Interaktion von zwei metabotropen Rezeptoren beeinflussen, für die Behandlung von Krankheiten, stellt unabhängig von dem speziellen hier für den GABA<sub>B</sub>-Rezeptor beschriebenen Fall ein neues pharmak-

kologisches Prinzip dar.

Dies ermöglicht ein Verfahren zum Auffinden von Substanzen mit spezifischer Bindungsaffinität zum erfindungsgemäßen Proteinheteromer bzw. Protein, das folgende Schritte umfaßt.

- a) Inkubation des oder der Proteine mit der zu testenden Substanz.
- b) Detektion der Bindung der zu testenden Substanz an das Protein.

Die Detektion der Bindung erfolgt durch Messen der Antagonisierung oder Agonisierung der GABA<sub>B</sub>-Rezeptor-Aktivität oder durch Messen einer physiologischen Wirkung, wie z. B. einer Änderung der Calcium-, cAMP-, IP<sub>3</sub>-Konzentration oder des Membranpotentials.

Weitere Ausgestaltungsformen der Erfindung sind ein Verfahren zum Auffinden von Substanzen, die die Interaktion von Proteinen mit Aminosäuresequenzen, wie sie in SEQ ID NO: 2 bzw. SEQ ID NO: 4 dargestellt werden, mit anderen metabotropen Rezeptoren hemmen oder verstärken; ein Verfahren zum Auffinden von Substanzen, die die Interaktion von Liganden mit dem erfindungsgemäßen Proteinheteromer oder dem erfindungsgemäßen Proteinen mit Aminosäuresequenzen wie SEQ ID NO: 2 bzw. SEQ ID NO: 4 hemmen oder verstärken oder ein Verfahren zum Auffinden von Substanzen, die die Interaktion von Proteinen mit Aminosäuresequenzen wie SEQ ID NO: 2 bzw. SEQ ID NO: 4 mit G-Proteinen oder anderen Signaltransduktionsmolekülen hemmen oder verstärken. Die Interaktion von Proteinen mit den erfindungsgemäßen Aminosäuren läßt sich mit Hilfe des Two-hybrid Systems detektieren. Weiter lassen sich die Verfahren durch Expression der Proteine in eukaryotischen Zellen und Verknüpfung mit einem Reporter-Assay für die Aktivierung des GABA<sub>B</sub>-Rezeptors durchführen. Dabei wird beispielsweise die Änderung des cAMP-Spiegels oder des Membranpotentials detektiert.

Weiterhin betrifft die Erfindung ein Verfahren zur qualitativen und quantitativen Bestimmung von Proteinen mit Aminosäuresequenzen wie SEQ ID NO: 2 oder SEQ ID NO: 4 unter Benutzung von spezifischen Agonisten oder Antagonisten. Dabei wird die GABA<sub>B</sub>-Rezeptor-Ligandenbindung zur Detektion ausgenutzt.

Über Antikörper kann die Proteinaktivität der Proteine mit den Sequenzen SEQ ID NO: 2 oder SEQ ID NO: 4 bestimmt werden. Daher ist ein weiterer Gegenstand der Erfindung ein Verfahren zur Quantifizierung der Proteinaktivität eines Proteins mit den Sequenzen SEQ ID NO: 2 oder SEQ ID NO: 4.

Die regulatorischen Sequenzen der erfindungsgemäßen Nukleinsäuren, insbesondere der Promotor, die Enhancer, locus control regions und silencer oder jeweilige Teilsequenzen davon können für die gewebespezifische Expression von diesem und weiteren Genen verwendet werden. Damit ergibt sich die Möglichkeit, gehirnspezifische Genexpression von Nukleinsäurekonstrukten durchzuführen.

Um ein DNA-Fragment zu isolieren, das die Bereiche enthält, die die Transkription der Sequenzen SEQ ID NO: 1 oder SEQ ID NO: 3 regulieren, wird zunächst eine genomische Bank mit einer möglichst weit 5'-positionierten cDNA-Probe durchsucht. Dazu wird eine dem Fachmann geläufige Homologiesuche durchgeführt (Ausubel et al. [eds.], 1998, Current Protocols in Molecular Biology, John Wiley & Sons, New York). Auf dem isolierten DNA-Fragment wird dann der Transkriptionsstart identifiziert. Der Bereich vor dem Transkriptionsstart wird anschließend mit einem Reportergen wie  $\beta$ -Galactosidase oder GFP (= green fluorescent protein) verknüpft und in Zellen oder in transgenen Tieren wie Mäusen daraufhin getestet, ob er zu dem für SEQ ID NO: 1 oder SEQ ID NO: 3 spezifischen Expressionsmuster führt (Ausubel et al. siehe oben). Das Reportergen kann anschließend mit anderen cDNAs verknüpft werden, um Tiermodelle zu erstellen, in denen die jeweilige cDNA regionspezifisch exprimiert wird (siehe beispielsweise Oberdick et al., Science, 248, 1990: 223-226).

Ausgehend von den Aminosäuresequenzen SEQ ID NO: 2 oder SEQ ID NO: 4 können synthetische Peptide generiert werden, die als Antigene für die Produktion von Antikörpern eingesetzt werden. Es ist auch möglich, das Polypeptid oder Bruchstücke davon zur Generierung von Antikörpern einzusetzen. Mit Antikörpern sind sowohl polyklonale, monoklonale, humane oder humanisierte oder rekombinante Antikörper oder Fragmente davon, single chain Antikörper oder auch synthetische Antikörper gemeint. Unter erfindungsgemäßen Antikörpern oder deren Fragmente sind prinzipiell alle Immunoglobulinklassen wie IgM, IgG, IgD, IgE, IgA oder ihre Subklassen wie die Subklassen des IgG oder deren Mischungen zu verstehen. Bevorzugt sind IgG und seine Subklassen wie beispielsweise IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>2a</sub>, IgG<sub>2b</sub>, IgG<sub>3</sub> oder IgG<sub>M</sub>. Besonders bevorzugt sind die IgG Subtypen IgG<sub>1</sub>/k oder IgG<sub>2b</sub>/k. Als Fragmente seien alle verkürzten oder veränderten Antikörperfragmente mit einer oder zwei dem Antigen-komplementären Bindungsstellen, wie Antikörperteile mit einer den Antikörper entsprechenden von leichter und schwerer Kette gebildeten Bindungsstelle wie Fv-, Fab- oder F(ab')<sub>2</sub>-Fragmente oder Einzelstrangfragmente, genannt. Bevorzugt sind verkürzte Doppelstrangfragmente wie Fv-, Fab- oder F(ab')<sub>2</sub>. Diese Fragmente können beispielsweise auf enzymatischem Wege durch Abspaltung des Fc-Teils der Antikörper mit Enzymen wie Papain oder Pepsin, durch chemische Oxidation oder durch gentechnische Manipulation der Antikörpergene erhalten werden. Auch genmanipulierte nichtverkürzte Fragmente können vorteilhaft verwendet werden.

Die Antikörper oder Fragmente können allein oder in Mischungen verwendet werden.

Die Antikörpergene für die gentechnischen Manipulationen lassen sich in dem Fachmann bekannterweise beispielsweise aus den Hybridomzellen isolieren (Harlow, E. and Lane, D. 1988, Antibodies: A Laboratory Manual, Cold Spring Harbor Press, N. Y.; Ausubel et al. [eds.], 1998, Current Protocols in Molecular Biology, John Wiley & Sons, New York). Dazu werden Antikörper-produzierende Zellen angezogen und die mRNA bei ausreichender optischer Dichte der Zellen über Zellyse mit Guanidiniumthiocyanat, Ansäuern mit Natriumacetat, Extraktion mit Phenol, Chloroform/Isoamylalkohol, Fällungen mit isopropanol und Waschen mit Ethanol aus den Zellen in bekannter Weise isoliert. Anschließend wird mit Hilfe der Reversen Transcriptase cDNA aus der mRNA synthetisiert. Die synthetisierte cDNA kann direkt oder nach genetischer Manipulation beispielsweise durch "site directed mutagenesis", Einführung von Insertionen, Inversionen, Deletionen oder Basenaustausche in geeignete tierische, pflanzliche, bakterielle oder virale Vektoren inseriert und in den entsprechenden Wirtsorganismen exprimiert werden. Bevorzugt werden bakterielle oder Hefe Vektoren wie pBR322, pUC18/19, pACYC184, Lambda oder Hefe-mu-Vektoren zur Klonierung der Gene und die Expression in Bakterien wie

E. coli bzw. in der Hefe wie *Saccharomyces cerevisiae*.

Spezifische Antikörper gegen die erfindungsgemäßen Proteine können sich sowohl als diagnostische Reagenzien als auch als Therapeutika bei neurologischen oder psychiatrischen Krankheitsbildern eignen.

Weiterhin können die cDNA, die genomische DNA, die regulatorischen Elemente der findungsgemäßen Nukleinsäuressequenzen, als auch das Polypeptid, sowie Teilfragmente davon in rekombinanter oder nichtrekombinanter Form zur Ausarbeitung eines Testsystems verwendet werden. Dieses Testsystem ist geeignet, die Aktivität des Promotors oder des Proteins in Anwesenheit der Testsubstanz zu messen. Bevorzugt handelt es sich hierbei um einfache Meßmethoden (kolorimetrische, luminometrische, auf Fluoreszenz beruhende oder radioaktive), die die schnelle Messung einer Vielzahl von Testsubstanzen erlauben (Böhm, Klebe, Kubinyi, 1996, Wirkstoffdesign, Spektrum-Verlag, Heidelberg). Die beschriebenen Testsysteme erlauben das Durchsuchen von chemischen Bibliotheken nach Substanzen, die agonistische oder antagonistische Wirkungen auf SEQ ID NO: 2 oder SEQ ID NO: 4 oder den neuen GABA<sub>B</sub>-Rezeptor-Komplex, bestehend aus dem bereits beschriebenen GABA<sub>B</sub>-Rezeptor und dem in SEQ ID NO: 2 oder SEQ ID NO: 4 beschriebenen Protein, haben. Die Identifizierung solcher Substanzen stellt den ersten Schritt auf dem Weg zur Identifizierung neuartiger Medikamente dar, die spezifisch auf die GABAerge Signaltransduktion wirken.

Ein alternativer Weg zur Entwicklung von Wirkstoffen, die am neuen GABA<sub>B</sub>-Rezeptor angreifen, besteht im rationalen Drug Design (Böhm, Klebe, Kubinyi, 1996, Wirkstoffdesign, Spektrum-Verlag, Heidelberg). Hier wird die Struktur oder eine Teilstruktur von dem in SEQ ID NO: 2 oder SEQ ID NO: 4 dargestellten Protein, soweit sie vorliegt, oder ein von Computern erstelltes Modell der Struktur, benutzt, um mit Unterstützung von Molecular Modelling Programmen Strukturen zu finden, für die sich eine hohe Affinität an den GABA<sub>B</sub>-Rezeptor vorhersagen läßt. Diese Substanzen werden dann synthetisiert und getestet. Hochaffine, selektive Substanzen werden auf ihre Verwendung als Medikamente gegen Epilepsie, Schlaganfall und andere neurologische Erkrankungen getestet.

Die Bestimmung von Menge, Aktivität und Verteilung des neuen GABA<sub>B</sub>-Rezeptor-Komplexes oder von dem in SEQ ID NO: 2 oder SEQ ID NO: 4 dargestellten Protein oder seiner zugrundeliegenden mRNA im menschlichen Körper kann zur Diagnose, Erfassung der Prädisposition und zum Monitoring bei bestimmten Erkrankungen dienen. Desgleichen kann die Sequenz der cDNA der Sequenzen SEQ ID NO: 2 oder SEQ ID NO: 4 sowie der genomischen DNA zu Aussagen über genetische Ursachen und Prädispositionen bestimmter Erkrankungen herangezogen werden. Dazu können sowohl DNA/RNA-Proben als auch Antikörper verschiedenster Art benutzt werden. Dabei dient die beschriebene Nukleotidsequenz SEQ ID NO: 1 oder SEQ ID NO: 3 oder Teile davon in Form geeigneter Proben zur Aufdeckung von Punktmutationen oder Deletionen/Insertionen/Rearrangements.

Die vorliegende Nukleinsäuresequenz SEQ ID NO: 1 oder SEQ ID NO: 3, ihre funktionellen Äquivalente, Homologe oder Derivate, das von ihr kodierte Protein (SEQ ID NO: 2 oder SEQ ID NO: 4) oder das erfindungsgemäße Proteinheteromer sowie davon abgeleitete Reagenzien (Oligonukleotide, Antikörper, Peptide) können zur Diagnose und Therapie von neurologischen Erkrankungen eingesetzt werden. Außerdem wird die Diagnose und Behandlung von genetischen Prädispositionen für bestimmte neurologische Erkrankungen wie Epilepsie, Schlaganfall, psychische Erkrankungen wie Angst, manisch-depressive Erkrankungen, Migräne, kognitive Verluste und weitere neurologische Erkrankungen möglich. Weiterhin kann ein Monitoring der Behandlung oben angegebener Erkrankungen durchgeführt werden.

Ein weiterer Gegenstand der Erfindung ist ein Verfahren zum qualitativen und quantitativen Nachweis einer erfindungsgemäßen Nukleinsäure in einer biologischen Probe, das folgende Schritte umfaßt:

- a) Inkubation einer biologischen Probe mit einer bekannten Menge an erfindungsgemäßer Nukleinsäure oder einer bekannten Menge an Oligonukleotiden, die als Primer für eine Amplifikation der erfindungsgemäßen Nukleinsäure geeignet sind,
- b) Nachweis der erfindungsgemäßen Nukleinsäure durch spezifische Hybridisierung oder PCR-Amplifikation,
- c) Vergleich der Menge an hybridisierender Nukleinsäure oder an durch PCR Amplifikation gewonnener Nukleinsäure mit einem Mengenstandard.

Außerdem betrifft die Erfindung ein Verfahren zum qualitativen und quantitativen Nachweis eines erfindungsgemäßen Proteinheteromers oder eines erfindungsgemäßen Proteins in einer biologischen Probe, das folgende Schritte umfaßt:

- a) Inkubation einer biologischen Probe mit einem Antikörper, der spezifisch gegen das Proteinheteromer oder gegen das erfindungsgemäße Protein gerichtet ist,
- b) Nachweis des Antikörper/Antigenkomplexes,
- c) Vergleich der Mengen des Antikörper/Antigenkomplexes mit einem Mengenstandard.

Als Standard wird üblicherweise eine biologische Probe aus einem gesunden Organismus entnommen.

Weiterhin betrifft die Erfindung ein Verfahren zum Auffinden von Substanzen, die spezifisch an ein Protein mit einer Aminosäuresequenz SEQ ID NO: 2 oder SEQ ID NO: 4 binden, das einen oder mehrere der folgenden Schritte umfaßt:

- a) Expression des Proteins in eukaryotischen oder prokaryotischen Zellen.
- b) Inkubation des Proteins mit den zu testenden Substanzen.
- c) Nachweis der Bindung einer Substanz an den Rezeptor bzw. eines Effektes auf die Rezeptorfunktion.

Außerdem betrifft die Erfindung ein Verfahren zum Auffinden von Substanzen, die spezifisch an ein Protein mit einer Aminosäuresequenz gemäß SEQ ID NO: 2 oder SEQ ID NO: 4 bzw. an eine Nukleinsäuresequenz gemäß SEQ ID NO: 1 oder SEQ ID NO: 3 binden und dadurch hemmende oder aktivierende funktionelle Effekte auf die GABAerge Signalweiterleitung in zentralnervösen Neuronen hervorrufen.

Je nach dem Neurotransmittersystem (z. B. GABA oder Glutamat), in das der GABA<sub>B</sub>-Rezeptor involviert ist, kann eine erhöhte oder eine erniedrigte GABA<sub>B</sub>-Rezeptor-Aktivität zu einem Ungleichgewicht zwischen den Neurotransmit-

tersystemen und oftmals zu einer neuronalen Übererregung führen, die eine Vielzahl neurologischer Erkrankungen charakterisiert, wie z. B. Epilepsie, Schlaganfall und deren Folgen und andere.

Mangelnde neuronale Aktivität, die z. B. Demenzen charakterisiert, kann die Folge sein, wenn präsynaptische GABA<sub>B</sub> Rezeptoren an glutamatergen Synapsen überaktiviert sind und die Transmitterausschüttung so stark hemmen, daß keine Reizweiterleitung über die Synapse mehr möglich ist.

In Situationen, in denen ein Mangel an der Aktivität des erfindungsgemäßen Proteins oder des mit SEQ ID NO: 2 oder SEQ ID NO: 4 komplementierten GABA<sub>B</sub>-Rezeptors herrscht, können mehrere Methoden zur Substituierung eingesetzt werden. Zum einen kann das Protein, natürlich oder rekombinant direkt, oder durch geeignete Maßnahmen in Form seiner kodierenden Nukleinsäure (d. h. DNA oder RNA) appliziert werden. Dazu können sowohl virale, als auch nichtvirale Vehikel zum Einsatz kommen. Ein weiterer Weg bietet sich durch die Stimulation des endogenen, körpereigenen Genes durch geeignete Substanzen. Solche Substanzen lassen sich beispielsweise auffinden, indem man ihre Wirkung auf die Transkriptionselemente des neuen GABA<sub>B</sub>-Rezeptor-Genes ermittelt.

In Situationen, in denen ein Überschuß an Aktivität des ein Protein mit der Sequenz SEQ ID NO: 2 oder SEQ ID NO: 4 beinhaltenden GABA<sub>B</sub>-Rezeptors oder von einem Protein mit SEQ ID NO: 2 oder SEQ ID NO: 4 allein herrscht, können spezifische, synthetische oder natürliche, kompetitive und nicht-kompetitive Antagonisten gegen das Protein mit der Sequenz SEQ ID NO: 2 oder SEQ ID NO: 4 oder Antikörper oder Antikörperfragmente gegen das Protein mit der Sequenz SEQ ID NO: 2 oder SEQ ID NO: 4 oder gegen das Proteinheteromer eingesetzt werden. Desweiteren kann sowohl durch antisense Moleküle oder Ribozyme oder Oligonukleotide als auch durch niedermolekulare Verbindungen eine Inhibition der GABA<sub>B</sub>-Rezeptor-Aktivität bzw. der Aktivität des Proteins mit der Sequenz SEQ ID NO: 2 oder SEQ ID NO: 4 erreicht werden.

### Beispiele

Die Nukleotidsequenz mit der in SEQ ID NO: 1 dargestellten Struktur wurde aus einer cDNA Bibliothek aus Ratten-Gehirn identifiziert. Bei der Suche nach Proteinen, die mit dem intrazellulären Carboxyterminus des bekannten GABA<sub>B</sub>-Rezeptors interagieren, wurde die Nukleotidsequenz gefunden. Im Experiment wurde eine cDNA-Bibliothek aus Ratten-Gehirn mit dem two-hybrid System nach Interaktionspartnern des Carboxyterminus des oben beschriebenen GABA<sub>B</sub>-Rezeptors durchsucht. Mehrere überlappende Fragmente einer unbekannten cDNA wurden gefunden. Mit Hilfe dieser Fragmente wurde aus einer cDNA-Bibliothek aus Ratten-Hippokampus ein 5kb langes Fragment der unbekannten cDNA durch Homologiescreening isoliert und anschließend sequenziert. Die so gewonnene cDNA-Sequenz enthält den kompletten kodierenden Bereich für die Sequenz SEQ ID NO: 2. Die Entdeckung und molekulare Charakterisierung von Interaktionspartnern des bekannten klonierten GABA<sub>B</sub>-Rezeptors ermöglicht es, die physiologischen Eigenschaften und die biochemische und pharmakologische Vielfalt des GABA<sub>B</sub>-Rezeptors besser zu verstehen, sowie neue konkrete Angriffspunkte für pharmakotherapeutische Interventionen zu erhalten.

Die Sequenzanalyse des durch die vorliegende cDNA (= SEQ ID NO: 1) kodierten Polypeptides läßt darauf schließen, daß es sich um einen metabotropen Rezeptor handelt. Es enthält eine aminoterminal Signalsequenz [Aminosäuren 1-40 (G. von Heijne, N. A. R., 14, 4683, 1986)] sowie sieben charakteristisch angeordnete hydrophobe Bereiche, die mit hoher Wahrscheinlichkeit die Plasmamembran durchspannen. Das Vorhandensein dieser sieben hydrophoben Bereiche ist ein Charakteristikum von metabotropen Rezeptoren. Sequenzvergleiche (Altschul et al., J. Mol. Biol., 215, 403-410, 1990) der Aminosäuresequenz SEQ ID NO: 2 mit den Sequenzen der offenen Leseraster der translatierten öffentlichen Nukleotiddatenbanken (nrdb) mit Hilfe des BLAST-Programmes (Version BLASTP 2.0a19-WashU [05-Feb-1998]) zeigten Ähnlichkeiten zu dem oben beschriebenen GABA<sub>B</sub>-Rezeptor (höchste Bewertung (best score): 36% Sequenzidentität über 804 Aminosäuren mit GABA<sub>B</sub>-Rezeptor 1B und 36% Sequenzidentität über 744 Aminosäuren mit GABA<sub>B</sub>-Rezeptor 1A) sowie eine wesentlich geringere Sequenzähnlichkeit zu metabotropen Glutamaterezeptoren und Calciumsensitiven Rezeptoren. Die Ähnlichkeit erstreckt sich über den aminoterminalen Bereich sowie besonders über die Transmembranregionen, aber nicht über den carboxyterminalen, intrazellulären Bereich (siehe Fig. 2). Bei dem in SEQ ID NO: 2 beschriebenen Protein handelt es sich demnach um einen neuen metabotropen Rezeptor, der entweder allein oder in Komplex mit dem bekannten GABA<sub>B</sub>-Rezeptor Signalweiterleitung im ZNS vermittelt oder diese reguliert.

Fig. 2 gibt einen Sequenzvergleich des erfindungsgemäßen Proteins mit der SEQ ID NO: 2 mit den bekannten GABA<sub>B</sub>-Rezeptor-Proteinen (= GBR) 1A und 1B (Kaupmann et al. 1997, siehe oben) wieder. Identische Aminosäuren sind durch schwarze, konservative Veränderungen durch hellgraue Schattierungen dargestellt. Die sieben Transmembranregionen (= TM1 bis TM7) sind durch darüberliegende Balken markiert.

Die Analyse der Verteilung der mRNA, von der die cDNA-Sequenz SEQ ID NO: 1 stammt, wurde mittels Northern Blot und mittels In-situ Hybridisierung auf Rattenhirnschnitten durchgeführt. Eine Analyse in 10 verschiedenen Ratten-Geweben ergab eine gehirnspezifische Expression einer 5-6 kb großen mRNA. In sehr geringem Umfang läßt sich eine kleinere mRNA in Ratten-Testis nachweisen (siehe Fig. 1). Die In-situ Hybridisierung ergab eine starke Expression in Hippokampus, Cortex, Cerebellum sowie in thalamischen Kernen. Das Expressionsmuster überlappt mit dem des GABA<sub>B</sub>-Rezeptors und deutet auf eine wichtige zentralnervöse Funktion des in SEQ ID NO: 2 dargestellten Proteins hin.

Soweit nicht anders angegeben wurde bei der experimentellen Durchführung entsprechend den Vorschriften in "Ausubel et al. (eds.), 1998. Current Protocols in Molecular Biology. John Wiley & Sons, New York" verfahren.

### Beispiel 1

#### Two-hybrid Suche mit dem Carboxyterminus von GABA<sub>B</sub>-Rezeptor 1

Die für den Carboxyterminus des GABA<sub>B</sub>-Rezeptor 1A kodierende cDNA (Aminosäuren 857-960, accession no. Y10369, EMBL-Datenbank) wurde mit den spezifischen Primern GABA-CT5' (5'-GCCGAATTCGACGGCTGAT-CACCCGAGGG-3') und GABA-CT3' (5'-GCAGTCGACTCACTTGTAAGCAAATGTACTCG-3') von Rattenhirn

cDNA in einer Polymerase-Ketten-Reaktion (PCR) amplifiziert, mit den Enzymen EcoRI und SalI einer Restriktion unterzogen und danach über die überhängenden Enden in einen mit den Enzymen EcoRI und SalI vorgeschrittenen Vektor pGBT (Firma Clontech) kloniert. Das so entstandene DNA-Konstrukt (pGBT-GABA<sub>B</sub>-Rezeptor 1) kodiert für ein Protein, in dem die Gal4-DNA-Bindungsdomäne mit dem C-terminus des GABA<sub>B</sub>-Rezeptors fusioniert ist. Mit diesem

5 Konstrukt wurde der Hefestamm HF7c (Firma Clontech) transformiert. Der resultierende Hefestamm wurde mit einer Rattenhirn cDNA Bank (Kornau et al., Science 269, 1737-1740, 1995) im Vektor pGAD (Firma Clontech) transformiert und  $4 \times 10^6$  Transformanten auf Tryptophan/Leucin/Histidin-Mangelmedium plattiert. Nach 3, 4 und 5 Tagen Wachstum bei 30°C wurden Kolonien mit einem Durchmesser von mehr als 2 mm vereinzelt und einer XGal-Färbung unterzogen. Insgesamt 7 Kolonien erwiesen sich als His3 und lacZ positiv (pGAD-pos1-7). Aus diesen wurde die jeweilige cDNA

10 aus dem Vektor pGAD mit dem vektorspezifischen Primer Gal4AD3' (5'-AAGAGATCCTAGAACTAGTGGATC-3') sowie T7 (5'-CGTAATACGACTCACTATAGGGCG-3') amplifiziert und das Amplikon sequenziert. Die Sequenzanalyse ergab 5 verschiedene überlappende Fragmente (Nukleotidsequenzen 2399 3102 (a) 2432 3102 (b), 2447 3102 (c), 2462-3102 (d), 2468-3102 (e) in der Sequenz SEQ ID NO: 1).

Aus zwei verschiedenen positiven Kolonien (a und e) wurde die pGAD-Plasmid-DNA aufgereinigt und mit verschiedenen pGBT-Konstrukten in den Hefestamm HF7c kotransformiert. Nur in Kombination mit dem Konstrukt pGBT-GABA<sub>B</sub>-Rezeptor 1 konnte eine Aktivierung der Reportergene His3 und lacZ festgestellt werden.

#### Beispiel 2

##### Klonierung der cDNA für die neue GABA<sub>B</sub>-Rezeptor-Komponente SEQ ID NO: 2

Ein aus der two-hybrid Suche gewonnenes cDNA-Fragment gemäß Beispiel 1 (a, Nukleotide 2399-3102 in Sequenz SEQ ID NO: 1) wurde mit dem random primed labelling kit (Boehringer Mannheim) entsprechend den Herstellerangaben mit  $\alpha$ -<sup>32</sup>P-dCTP radioaktiv markiert. Die durch Erhitzen denaturierte radioaktive Sonde wurde auf 18 Nitrocellulose-

25 Filter, auf die je 40000 Plaques einer cDNA-Bank aus Ratten-Hippokampus im Bakteriophagen  $\lambda$  transferiert worden waren, 16 Stunden lang hybridisiert (42°C,  $5 \times$  SSC, 50% Formamid) und daraufhin mehrfach mit  $0,2 \times$  SSC, 60°C gewaschen. Von 30 positiven  $\lambda$ -Klonen wurden 6 vereinzelt, Phagen-DNA isoliert und kartiert. Die beiden längsten cDNA-Fragmente (5kb) wurden vollständig sequenziert. Sie enthalten ein offenes Leseraster für die Aminosäuresequenz SEQ ID NO: 2. Die Sequenzanalyse ergab bei diesen beiden Lambda-cDNA-Klonen vier Unterschiede in der kodierenden Sequenz, von denen 3 stille Mutationen darstellen (Nukleotid 696 C zu T, Nukleotid 1104 T zu C, Nukleotid 2295 C zu T) und eine für ein zusätzliches Prolin in der aminoterminalen Signalsequenz kodiert (Insertion von CCG bei Nukleotid 171/172).

#### Beispiel 3

##### Expression der mRNA für die neue GABA<sub>B</sub>-Rezeptor-Komponente in Ratten-Gewebe

Ein aus der two-hybrid Suche gewonnenes cDNA-Fragment gemäß Beispiel 1 (a, Nukleotide 2399 3102 in SEQ ID NO: 1) wurde mit dem random primed labelling kit (Boehringer Mannheim) entsprechend den Herstellerangaben mit  $\alpha$ -<sup>32</sup>P-dCTP radioaktiv markiert. Die durch Erhitzen denaturierte radioaktive Sonde wurde mit einem Multiple Tissue Northern Blot (je 10  $\mu$ g total RNA von Ratten-Gehirn, -Leber, -Lunge, -Herz, -Niere, -Hoden, -Muskel und -Darm, isoliert nach "Chomczynski and Sacchi, Anal. Biochem., 162, 156-159, 1987") in QuickHyb-Lösung (Firma Stratagene) für eine Stunde bei 68°C hybridisiert und daraufhin bei 60°C und  $0,1 \times$  SSC gewaschen. Nach drei Tagen Exposition wurde ein starkes Hybridisierungssignal auf Gehirn-RNA bei etwa 5-6 kb, ein wesentlich schwächeres Signal geringerer Größe auf

45 Hoden-RNA und kein Signal in allen anderen untersuchten Geweben festgestellt (siehe Fig. 1a).

#### Beispiel 4

##### Expression der mRNA für die neue GABA<sub>B</sub>-Rezeptor-Komponente im Ratten-Gehirn

Wenn nicht anders beschrieben, wurde die in-situ Hybridisierung durchgeführt wie in "Molecular Neurobiology: A Practical Approach. J. Chad and H. Wheat, eds. (Oxford: IRL Press), pp.205-225" beschrieben.

Zwei Antisense-Oligonukleotide (revers komplementär zu den Nukleotiden 2463-2498 bzw. Nukleotiden 2538-2573 in der Sequenz SEQ ID NO: 1) wurden mit Terminaler Desoxynukleotid-Transferase (Boehringer Mannheim) entsprechend den Herstellerangaben mit  $\alpha$ -<sup>35</sup>S-dATP radioaktiv markiert. Diese radioaktiven Sonden wurden auf etwa 15  $\mu$ m dicke horizontale Rattenhirnschnitte appliziert und 16 Stunden lang in  $4 \times$  SSC, 50% Formamid bei 42°C hybridisiert. Daraufhin wurden die Schnitte für 30 Minuten in  $1 \times$  SSC, 55°C gewaschen und für 8 Tage exponiert. Die Autoradiogramme für beide Oligonukleotide zeigten ein übereinstimmendes Bild (siehe Fig. 1b). Das stärkste Signal wurde in Purkinje-Zellen des Kleinhirns, ebenfalls starke Signale in Kortex, Hippokampus und in verschiedenen thalamischen Kernen sowie schwächere Signale in den Körnerzellen des Kleinhirns gefunden.

#### Beispiel 5

##### Klonierung und Sequenzierung der cDNA für die humane Form der neuen GABA<sub>B</sub>-Rezeptorkomponente SEQ ID NO: 3 (DNA-Sequenz) bzw. SEQ ID NO: 4 (Aminosäuresequenz)

Ausgehend von 5  $\mu$ g totaler RNA aus dem Gesamthirn eines 57 Jahre alten Mannes (Firma Clontech) wurde mit Superscript Reverser Transkriptase (Gibco BRL) entsprechend den Hinweisen des Herstellers cDNA synthetisiert. Mit

mehreren Oligonukleotidprimern, die von SEQ ID NO: 1 bzw. von in der EMBL-Datenbank befindlichen humanen EST-Sequenzen abgeleitet wurden, konnten in RCR-Reaktionen von der humanen cDNA spezifische Produkte amplifiziert werden. Hierbei handelte es sich um folgende Primer-Paare:

GB1s/GB6as 5  
GB15s(hs)/GB18as  
GB17s/GB11as  
GB17s/GB16as(hs)  
GB25s/GB4as  
GB25s/GB23as(hs) 10  
GB25sXbaI/GB23as(hs)  
GB22s(hs)/GB16as(hs)

GB1s: 5'-CAGATCCGCAACGAGTCACTCCTG-3'  
GB2as: 5'-CAGGAGTGACTCGTTGCGGATCTG-3' 15  
GB3s: 5'-CAGTTTGACCAGAATATGGCAGC-3'  
GB4as: 5'-GC'TGCCATATCTGGTCAAACCTG-3'  
GB6as: 5'-GACCTTCACCTCTCTGCTGTCTTG-3'  
GB11as: 5'-GAAGGAGGGTGGTACGTGTCTGTG-3'  
GB15s(hs): 5'-CTACGATGGCATCTGGGTCATC-3' 20  
GB16as(hs): 5'-GTCCCATTTCGTTCTCTCTTC-3'  
GB17s: 5'-CTCAACGACAGCAAGTACATC-3'  
GB18as: 5'-GATGTACTTGCTGTCTGTTGAG-3'  
GB19as(hs): 5'-GCTCTAGACCGTATTTATTGCATCGTAG-3'  
GB22s(hs): 5'-GCGAATTCACAAAAAGACAAGACCATCATCCTG-3' 25  
GB23as(hs): 5'-GCGAATTCAGGATGGTGAGGGCAGAGAGGATG-3'  
GB25s: 5'-GTGAATTCGCGGCGCGGCATGGCTTC-3'  
GB25sXbaI: 5'-GT'TCTAGACGCGGCGCGGCATGGCTTC-3'  
GB27as: 5'-CTGGTCCCCGGGTCAGGAAGGAGAC-3' 30

Die PCR-Produkte wurden direkt mit den Primern, die schon zur Amplifikation benutzt worden waren bzw. mit den oben genannten Primern, sequenziert. Aus den erhaltenen Sequenzen wurden neue Primer abgeleitet und für die PCR-Reaktionen und Sequenzierungen eingesetzt (Liste der Primer siehe oben). Aus den Einzelsequenzen der erhaltenen PCR-Produkte konnte schließlich die Sequenz SEQ ID NO: 3 zusammengesetzt werden, die das offene Leseraster für ein Protein der Aminosäuresequenz SEQ ID NO: 4 enthält. 35

An den Positionen 360 und 2605 in der Sequenz SEQ ID NO: 3 wurde nach wiederholtem Sequenzieren aus beiden Richtungen neben dem Aauch ein G-Signal detektiert, so daß an dieser Stelle die Sequenz entweder A oder G lautet. Diese Änderung gegenüber SEQ ID NO: 3 würde für Position 360 zu keiner Änderung der Aminosäuresequenz SEQ ID NO: 4 führen; an Position 2605 würde es zu einem Threoninzu Alanin-Austausch an Position 869 der Sequenz SEQ ID NO: 4 führen. Die Basenpaare 1 bis 8 in SEQ ID NO: 3 wurden durch den von 1 SEQ ID NO: 1 abgeleiteten Primer GB25s vorgegeben. Es kann daher nicht ausgeschlossen werden, daß eine oder mehrere Positionen in den Positionen 1 bis 8 in der SEQ ID NO: 3 anders lauten als angegeben. Demzufolge kann auch eine oder mehrere der Aminosäuren 1 bis 3 in SEQ ID NO: 4 anders lauten als angegeben. 40

Das PCR-Produkt GB25sXbaI/GB23as(hs) wurde einem Restriktionsverdau mit XbaI und BglII unterzogen. Das PCR-Produkt GB22s(hs)/GB16as(hs) wurde einem Restriktionsverdau mit BglII und XhoI unterzogen. Die beiden PCR-Produkte wurden in einen mit XbaI und XhoI vorgeschnittenen pBSIKS(-)-Vektor (Firma Stratagene) kloniert (pBS-hsGB). 45

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# DE 198 41 941 A 1

## SEQUENZPROTOKOLL

### (1) ALGEMEINE INFORMATION:

- 5 (i) ANMELDER:  
 (A) NAME: BASF-LYNX Bioscience AG  
 (B) STRASSE: Im Neuenheimer Feld 519  
 10 (C) ORT: Heidelberg  
 (D) BUNDESLAND: Baden-Wuerttemberg  
 (E) LAND: Deutschland  
 (F) POSTLEITZAHL: 69120
- 15 (ii) ANMELDETITEL: Neuer metabotroper GABA-Rezeptor-Komplex aus dem zentralen Nervensystem
- 20 (iii) ANZAHL DER SEQUENZEN: 4
- (iv) COMPUTER-LESBARE FORM:  
 (A) DATENTRÄGER: Floppy disk  
 (B) COMPUTER: IBM PC compatible  
 25 (C) BETRIEBSSYSTEM: PC-DOS/MS-DOS  
 (D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPA)

### 30 (2) INFORMATION ZU SEQ ID NO: 1:

- (i) SEQUENZ CHARAKTERISTIKA:  
 (A) LÄNGE: 3288 Basenpaare  
 (B) ART: Nukleinsäure  
 35 (C) STRANGFORM: Einzel  
 (D) TOPOLOGIE: linear
- (ii) ART DES MOLEKÜLS: cDNS zu mRNS
- 40 (iii) HYPOTHETISCH: NEIN
- (iii) ANTISENSE: NEIN
- 45 (vi) URSPRÜNGLICHE HERKUNFT:  
 (A) ORGANISMUS: Rattus norvegicus
- (ix) MERKMALE:  
 50 (A) NAME/SCHLÜSSEL: CDS  
 (B) LAGE: 118..2940

### (xi) SEQUENZBESCHREIBUNG: SEQ ID NO: 1:

55 CCGGGGCCCC TGCCGGCGCC ATTGCGCGGG AGCCGCGGGC AAAGCTCGGC GCCGGGCGGC 60

GGGCCGGGCC AGGCCATGCG GGCCGAGTGA GCTGGCGCCC GCAGCCCGCG GCGCGGC 117

60 ATG GCT TCC CCG CCG AGC TCC GGG CAG CCC CGG CCG CCG CCG CCG CCG 165

Met Ala Ser Pro Pro Ser Ser Gly Gln Pro Arg Pro Pro Pro Pro Pro

1 5 10 15

65

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CCG CCG CCC GCG CGC CTG CTG CTG CCC CTG CTG CTG TCG CTG CTG CTG	213
Pro Pro Pro Ala Arg Leu Leu Leu Pro Leu Leu Leu Ser Leu Leu Leu	
20 25 30	5
TGG TTG GCG CCC GGG GCC TGG GGC TGG ACG CGG GGC GCC CCC CGG CCG	261
Trp Leu Ala Pro Gly Ala Trp Gly Trp Thr Arg Gly Ala Pro Arg Pro	
35 40 45	10
CCG CCC AGC AGC CCG CCG CTC TCC ATC ATG GGC CTC ATG CCG CTC ACC	309
Pro Pro Ser Ser Pro Pro Leu Ser Ile Met Gly Leu Met Pro Leu Thr	
50 55 60	15
AAG GAG GTG GCC AAG GGC AGC ATC GGG CGC GGC GTG CTC CCC GCC GTG	357
Lys Glu Val Ala Lys Gly Ser Ile Gly Arg Gly Val Leu Pro Ala Val	
65 70 75 80	20
GAG CTA GCC ATC GAG CAG ATC CGC AAC GAG TCA CTC CTG CGC CCC TAC	405
Glu Leu Ala Ile Glu Gln Ile Arg Asn Glu Ser Leu Leu Arg Pro Tyr	
85 90 95	25
TTC CTG GAC CTG CGA CTC TAT GAC ACC GAG TGT GAC AAT GCA AAG GGA	453
Phe Leu Asp Leu Arg Leu Tyr Asp Thr Glu Cys Asp Asn Ala Lys Gly	
100 105 110	30
CTG AAA GCC TTC TAT GAC GCA ATA AAG TAT GGG CCG AAC CAT TTG ATG	501
Leu Lys Ala Phe Tyr Asp Ala Ile Lys Tyr Gly Pro Asn His Leu Met	
115 120 125	35
GTG TTT GGA GGC GTC TGT CCG TCT GTC ACA TCT ATT ATC GCG GAG TCC	549
Val Phe Gly Gly Val Cys Pro Ser Val Thr Ser Ile Ile Ala Glu Ser	
130 135 140	40
CTC CAA GGC TGG AAT CTG GTG CAG CTT TCC TTC GCC GCC ACC ACG CCT	597
Leu Gln Gly Trp Asn Leu Val Gln Leu Ser Phe Ala Ala Thr Thr Pro	
145 150 155 160	45
GTT CTT GCG GAT AAG AAG AAG TAC CCG TAT TTC TTC CGG ACG GTG CCG	645
Val Leu Ala Asp Lys Lys Lys Tyr Pro Tyr Phe Phe Arg Thr Val Pro	
165 170 175	50
TCA GAC AAC GCG GTG AAC CCC GCC ATC CTG AAG CTC CTG AAG CAC TTC	693
Ser Asp Asn Ala Val Asn Pro Ala Ile Leu Lys Leu Leu Lys His Phe	
180 185 190	55
CGC TGG CGG CGT GTG GGC ACA CTC ACG CAG GAC GTG CAG CGC TTC TCC	741
Arg Trp Arg Arg Val Gly Thr Leu Thr Gln Asp Val Gln Arg Phe Ser	
195 200 205	60
GAG GTG AGG AAT GAC CTG ACT GGG GTT CTG TAT GGG GAA GAT ATT GAG	789
Glu Val Arg Asn Asp Leu Thr Gly Val Leu Tyr Gly Glu Asp Ile Glu	
210 215 220	65

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	ATC	TCA	GAC	ACA	GAG	AGC	TTC	TCC	AAT	GAT	CCC	TGC	ACC	AGC	GTC	AAA	837
	Ile	Ser	Asp	Thr	Glu	Ser	Phe	Ser	Asn	Asp	Pro	Cys	Thr	Ser	Val	Lys	
5	225					230					235					240	
	AAG	CTC	AAG	GGG	AAT	GAC	GTG	CGG	ATC	ATC	CTT	GGC	CAG	TTT	GAC	CAG	885
	Lys	Leu	Lys	Gly	Asn	Asp	Val	Arg	Ile	Ile	Leu	Gly	Gln	Phe	Asp	Gln	
					245					250					255		
10	AAT	ATG	GCA	GCA	AAA	GTC	TTC	TGT	TGT	GCC	TTC	GAG	GAG	AGC	ATG	TTT	933
	Asn	Met	Ala	Ala	Lys	Val	Phe	Cys	Cys	Ala	Phe	Glu	Glu	Ser	Met	Phe	
					260					265					270		
15	GGC	AGC	AAG	TAC	CAG	TGG	ATC	ATC	CCG	GGA	TGG	TAC	GAG	CCT	GCG	TGG	981
	Gly	Ser	Lys	Tyr	Gln	Trp	Ile	Ile	Pro	Gly	Trp	Tyr	Glu	Pro	Ala	Trp	
			275					280					285				
20	TGG	GAG	CAG	GTG	CAT	GTG	GAG	GCC	AAT	TCC	TCA	CGC	TGC	CTG	CGC	AGA	1029
	Trp	Glu	Gln	Val	His	Val	Glu	Ala	Asn	Ser	Ser	Arg	Cys	Leu	Arg	Arg	
		290					295					300					
25	AGC	CTC	CTG	GCT	GCC	ATG	GAA	GGT	TAC	ATC	GGA	GTG	GAC	TTT	GAG	CCC	1077
	Ser	Leu	Leu	Ala	Ala	Met	Glu	Gly	Tyr	Ile	Gly	Val	Asp	Phe	Glu	Pro	
	305					310					315					320	
30	CTG	AGC	TCC	AAA	CAA	ATC	AAG	ACC	ATT	TCA	GGG	AAG	ACT	CCA	CAG	CAG	1125
	Leu	Ser	Ser	Lys	Gln	Ile	Lys	Thr	Ile	Ser	Gly	Lys	Thr	Pro	Gln	Gln	
					325					330					335		
35	TAT	GAA	AGA	GAG	TAC	AAC	AGC	AAA	CGT	TCA	GGC	GTG	GGG	CCC	AGC	AAG	1173
	Tyr	Glu	Arg	Glu	Tyr	Asn	Ser	Lys	Arg	Ser	Gly	Val	Gly	Pro	Ser	Lys	
				340					345					350			
40	TTC	CAT	GGG	TAC	GCC	TAC	GAT	GGG	ATC	TGG	GTC	ATC	GCC	AAG	ACC	CTA	1221
	Phe	His	Gly	Tyr	Ala	Tyr	Asp	Gly	Ile	Trp	Val	Ile	Ala	Lys	Thr	Leu	
			355					360					365				
45	CAG	AGG	GCC	ATG	GAG	ACA	CTG	CAT	GCC	AGT	AGC	AGG	CAC	CAG	CGG	ATC	1269
	Gln	Arg	Ala	Met	Glu	Thr	Leu	His	Ala	Ser	Ser	Arg	His	Gln	Arg	Ile	
		370					375					380					
50	CAG	GAC	TTC	AAC	TAC	ACA	GAC	CAC	ACG	CTG	GGC	AAA	ATC	ATC	CTC	AAT	1317
	Gln	Asp	Phe	Asn	Tyr	Thr	Asp	His	Thr	Leu	Gly	Lys	Ile	Ile	Leu	Asn	
	385					390					395				400		
55	GCC	ATG	AAC	GAG	ACC	AAC	TTC	TTC	GGG	GTC	ACG	GGT	CAA	GTT	GTG	TTC	1365
	Ala	Met	Asn	Glu	Thr	Asn	Phe	Phe	Gly	Val	Thr	Gly	Gln	Val	Val	Phe	
					405					410					415		
60	CGG	AAC	GGG	GAG	AGA	ATG	GGA	ACC	ATT	AAA	TTT	ACT	CAA	TTT	CAA	GAC	1413
	Arg	Asn	Gly	Glu	Arg	Met	Gly	Thr	Ile	Lys	Phe	Thr	Gln	Phe	Gln	Asp	
				420					425						430		

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AGC AGA GAG GTG AAG GTC GGC GAA TAC AAC GCG GTG GCT GAC ACA CTG Ser Arg Glu Val Lys Val Gly Glu Tyr Asn Ala Val Ala Asp Thr Leu 435 440 445	1461	5
GAG ATC ATC AAT GAC ACC ATA AGG TTC CAG GGG TCC GAG CCA CCC AAG Glu Ile Ile Asn Asp Thr Ile Arg Phe Gln Gly Ser Glu Pro Pro Lys 450 455 460	1509	10
GAC AAG ACC ATC ATT CTG GAG CAG CTT CGG AAG ATC TCG CTT CCA CTG Asp Lys Thr Ile Ile Leu Glu Gln Leu Arg Lys Ile Ser Leu Pro Leu 465 470 475 480	1557	15
TAT AGC ATC CTG TCC GCT CTC ACC ATC CTC GGC ATG ATC ATG GCC AGC Tyr Ser Ile Leu Ser Ala Leu Thr Ile Leu Gly Met Ile Met Ala Ser 485 490 495	1605	20
GCC TTC CTC TTC TTC AAC ATC AAG AAC CGG AAC CAA AAG CTG ATT AAG Ala Phe Leu Phe Phe Asn Ile Lys Asn Arg Asn Gln Lys Leu Ile Lys 500 505 510	1653	25
ATG TCA AGC CCC TAC ATG AAC AAC CTC ATC ATC CTG GGA GGA ATG CTG Met Ser Ser Pro Tyr Met Asn Asn Leu Ile Ile Leu Gly Gly Met Leu 515 520 525	1701	30
TCC TAT GCA TCC ATC TTC CTC TTT GGC CTC GAT GGG TCC TTC GTC TCA Ser Tyr Ala Ser Ile Phe Leu Phe Gly Leu Asp Gly Ser Phe Val Ser 530 535 540	1749	35
GAA AAG ACC TTT GAA ACA CTC TGC ACG GTC CGG ACC TGG ATT CTC ACC Glu Lys Thr Phe Glu Thr Leu Cys Thr Val Arg Thr Trp Ile Leu Thr 545 550 555 560	1797	40
GTG GGC TAC ACA ACT GCC TTT GGG GCC ATG TTT GCA AAG ACC TGG AGG Val Gly Tyr Thr Thr Ala Phe Gly Ala Met Phe Ala Lys Thr Trp Arg 565 570 575	1845	45
GTC CAT GCC ATC TTC AAA AAT GTG AAG ATG AAG AAG AAG ATC ATC AAA Val His Ala Ile Phe Lys Asn Val Lys Met Lys Lys Lys Ile Ile Lys 580 585 590	1893	50
GAC CAG AAG CTG CTT GTG ATT GTG GGG GGC ATG CTG CTC ATC GAC CTG Asp Gln Lys Leu Leu Val Ile Val Gly Gly Met Leu Leu Ile Asp Leu 595 600 605	1941	55
TGC ATC CTG ATC TGT TGG CAG GCT GTG GAC CCC CTG CGG AGG ACA GTG Cys Ile Leu Ile Cys Trp Gln Ala Val Asp Pro Leu Arg Arg Thr Val 610 615 620	1989	60
GAG AGG TAC AGC ATG GAG CCG GAC CCA GCA GGC CGG GAC ATC TCC ATC Glu Arg Tyr Ser Met Glu Pro Asp Pro Ala Gly Arg Asp Ile Ser Ile 625 630 635 640	2037	65

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	CGC CCA TTG CTG GAA CAC TGC GAA AAC ACC CAC ATG ACC ATC TGG CTT	2085
	Arg Pro Leu Leu Glu His Cys Glu Asn Thr His Met Thr Ile Trp Leu	
	645 650 655	
5		
	GGC ATT GTC TAC GCC TAC AAG GGG CTC CTC ATG CTA TTC GGT TGT TTC	2133
	Gly Ile Val Tyr Ala Tyr Lys Gly Leu Leu Met Leu Phe Gly Cys Phe	
	660 665 670	
10		
	TTG GCA TGG GAA ACC CGC AAT GTG AGC ATC CCT GCC CTC AAC GAC AGC	2181
	Leu Ala Trp Glu Thr Arg Asn Val Ser Ile Pro Ala Leu Asn Asp Ser	
	675 680 685	
15		
	AAG TAC ATC GGC ATG AGT GTG TAC AAT GTG GGG ATC ATG TGC ATC ATC	2229
	Lys Tyr Ile Gly Met Ser Val Tyr Asn Val Gly Ile Met Cys Ile Ile	
	690 695 700	
20		
	GGG GCT GCT GTC TCC TTC CTG ACG CGT GAC CAG CCC AAC GTG CAG TTC	2277
	Gly Ala Ala Val Ser Phe Leu Thr Arg Asp Gln Pro Asn Val Gln Phe	
	705 710 715 720	
25		
	TGC ATC GTG GCC CTG GTC ATC ATC TTC TGC AGC ACC ATC ACT CTC TGC	2325
	Cys Ile Val Ala Leu Val Ile Ile Phe Cys Ser Thr Ile Thr Leu Cys	
	725 730 735	
30		
	CTG GTG TTT GTG CCA AAG CTC ATC ACT CTG AGG ACA AAC CCT GAC GCA	2373
	Leu Val Phe Val Pro Lys Leu Ile Thr Leu Arg Thr Asn Pro Asp Ala	
	740 745 750	
35		
	GCC ACT CAG AAC AGG CGG TTC CAG TTC ACA CAG AAC CAG AAG AAA GAA	2421
	Ala Thr Gln Asn Arg Arg Phe Gln Phe Thr Gln Asn Gln Lys Lys Glu	
	755 760 765	
40		
	GAT TCG AAG ACC TCC ACT TCA GTC ACC AGC GTG AAC CAG GCG AGC ACG	2469
	Asp Ser Lys Thr Ser Thr Ser Val Thr Ser Val Asn Gln Ala Ser Thr	
	770 775 780	
45		
	TCA CGC CTG GAG GGA CTG CAG TCA GAA AAC CAC CGC CTT CGA ATG AAG	2517
	Ser Arg Leu Glu Gly Leu Gln Ser Glu Asn His Arg Leu Arg Met Lys	
	785 790 795 800	
50		
	ATC ACA GAG CTG GAC AAA GAC TTG GAA GAA GTC ACC ATG CAG CTA CAA	2565
	Ile Thr Glu Leu Asp Lys Asp Leu Glu Glu Val Thr Met Gln Leu Gln	
	805 810 815	
55		
	GAC ACA CCA GAG AAG ACC ACA TAC ATC AAA CAG AAT CAC TAC CAA GAG	2613
	Asp Thr Pro Glu Lys Thr Thr Tyr Ile Lys Gln Asn His Tyr Gln Glu	
	820 825 830	
60		
	CTC AAC GAC ATC CTC AGC TTG GGC AAC TTC ACA GAG AGC ACA GAT GGA	2661
	Leu Asn Asp Ile Leu Ser Leu Gly Asn Phe Thr Glu Ser Thr Asp Gly	
	835 840 845	

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GGA AAG GCC ATT CTA AAA AAT CAC CTC GAT CAA AAC CCC CAG CTC CAG	2709
Gly Lys Ala Ile Leu Lys Asn His Leu Asp Gln Asn Pro Gln Leu Gln	
850 855 860	5
TGG AAC ACG ACA GAG CCC TCA AGA ACA TGC AAA GAC CCC ATA GAA GAC	2757
Trp Asn Thr Thr Glu Pro Ser Arg Thr Cys Lys Asp Pro Ile Glu Asp	
865 870 875 880	10
ATC AAC TCC CCG GAG CAC ATC CAG CGC CGG CTG TCG CTC CAG CTC CCC	2805
Ile Asn Ser Pro Glu His Ile Gln Arg Arg Leu Ser Leu Gln Leu Pro	
885 890 895	15
ATC CTT CAC CAC GCC TAC CTC CCA TCC ATC GGA GGC GTG GAT GCC AGC	2853
Ile Leu His His Ala Tyr Leu Pro Ser Ile Gly Gly Val Asp Ala Ser	
900 905 910	20
TGC GTC AGC CCC TGT GTC AGC CCT ACC GCC AGC CCT CGC CAC AGA CAC	2901
Cys Val Ser Pro Cys Val Ser Pro Thr Ala Ser Pro Arg His Arg His	
915 920 925	25
GTA CCA CCC TCC TTC CGA GTC ATG GTC TCG GGC CTG TAGGGGTGGG	2947
Val Pro Pro Ser Phe Arg Val Met Val Ser Gly Leu	
930 935 940	30
AGGCCTGGGG CCGGGGCCTC CCCGGGACAG CACCATGCTG GGCCAAGGCG CCTGCCACAG	3007
GCACACTGAC GCGGGCGAGA AGCTGGGCAC CATGCTGCCT CTCCAGACTG CTGGAATGGC	3067
GCTCAGGCAG AGCGGGACTC GGCACCGACC TCGAGCCTTA TCTGTGAAGG TCTTACTCTC	3127
ACAGAGGAGA GGAATGACAA TGACTTCTCC TTCTTGCGGT CTGCAAACAA AGAGGAGTTG	3187
GGATGTCTGA AACTTGCAAA AACAAATCAA ACTCTAGACA AAGGAGAGAG GCCTCGGACT	3247
CCTGCTGTCC TCGCCAAGTG GCCAGAGCAA GGGCTCTGCA G	3288

## (2) INFORMATION ZU SEQ ID NO: 2:

### (i) SEQUENZ CHARAKTERISTIKA:

- (A) LÄNGE: 940 Aminosäuren
- (B) ART: Aminosäure
- (D) TOPOLOGIE: linear

### (ii) ART DES MOLEKÜLS: Protein

### (xi) SEQUENZBESCHREIBUNG: SEQ ID NO: 2:

Met Ala Ser Pro Pro Ser Ser Gly Gln Pro Arg Pro Pro Pro Pro	55
1 5 10 15	
Pro Pro Pro Ala Arg Leu Leu Leu Pro Leu Leu Leu Ser Leu Leu Leu	60
20 25 30	

65

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Trp Leu Ala Pro Gly Ala Trp Gly Trp Thr Arg Gly Ala Pro Arg Pro  
 35 40 45  
 5 Pro Pro Ser Ser Pro Pro Leu Ser Ile Met Gly Leu Met Pro Leu Thr  
 50 55 60  
 10 Lys Glu Val Ala Lys Gly Ser Ile Gly Arg Gly Val Leu Pro Ala Val  
 65 70 75 80  
 Glu Leu Ala Ile Glu Gln Ile Arg Asn Glu Ser Leu Leu Arg Pro Tyr  
 85 90 95  
 15 Phe Leu Asp Leu Arg Leu Tyr Asp Thr Glu Cys Asp Asn Ala Lys Gly  
 100 105 110  
 20 Leu Lys Ala Phe Tyr Asp Ala Ile Lys Tyr Gly Pro Asn His Leu Met  
 115 120 125  
 Val Phe Gly Gly Val Cys Pro Ser Val Thr Ser Ile Ile Ala Glu Ser  
 25 130 135 140  
 Leu Gln Gly Trp Asn Leu Val Gln Leu Ser Phe Ala Ala Thr Thr Pro  
 145 150 155 160  
 30 Val Leu Ala Asp Lys Lys Lys Tyr Pro Tyr Phe Phe Arg Thr Val Pro  
 165 170 175  
 35 Ser Asp Asn Ala Val Asn Pro Ala Ile Leu Lys Leu Leu Lys His Phe  
 180 185 190  
 Arg Trp Arg Arg Val Gly Thr Leu Thr Gln Asp Val Gln Arg Phe Ser  
 40 195 200 205  
 Glu Val Arg Asn Asp Leu Thr Gly Val Leu Tyr Gly Glu Asp Ile Glu  
 210 215 220  
 45 Ile Ser Asp Thr Glu Ser Phe Ser Asn Asp Pro Cys Thr Ser Val Lys  
 225 230 235 240  
 Lys Leu Lys Gly Asn Asp Val Arg Ile Ile Leu Gly Gln Phe Asp Gln  
 50 245 250 255  
 Asn Met Ala Ala Lys Val Phe Cys Cys Ala Phe Glu Glu Ser Met Phe  
 260 265 270  
 55 Gly Ser Lys Tyr Gln Trp Ile Ile Pro Gly Trp Tyr Glu Pro Ala Trp  
 275 280 285  
 60 Trp Glu Gln Val His Val Glu Ala Asn Ser Ser Arg Cys Leu Arg Arg  
 290 295 300  
 Ser Leu Leu Ala Ala Met Glu Gly Tyr Ile Gly Val Asp Phe Glu Pro  
 65 305 310 315 320



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Leu Ser Ser Lys Gln Ile Lys Thr Ile Ser Gly Lys Thr Pro Gln Gln	325	330	335	
Tyr Glu Arg Glu Tyr Asn Ser Lys Arg Ser Gly Val Gly Pro Ser Lys	340	345	350	5
Phe His Gly Tyr Ala Tyr Asp Gly Ile Trp Val Ile Ala Lys Thr Leu	355	360	365	10
Gln Arg Ala Met Glu Thr Leu His Ala Ser Ser Arg His Gln Arg Ile	370	375	380	15
Gln Asp Phe Asn Tyr Thr Asp His Thr Leu Gly Lys Ile Ile Leu Asn	385	390	395	400
Ala Met Asn Glu Thr Asn Phe Phe Gly Val Thr Gly Gln Val Val Phe	405	410	415	20
Arg Asn Gly Glu Arg Met Gly Thr Ile Lys Phe Thr Gln Phe Gln Asp	420	425	430	25
Ser Arg Glu Val Lys Val Gly Glu Tyr Asn Ala Val Ala Asp Thr Leu	435	440	445	30
Glu Ile Ile Asn Asp Thr Ile Arg Phe Gln Gly Ser Glu Pro Pro Lys	450	455	460	35
Asp Lys Thr Ile Ile Leu Glu Gln Leu Arg Lys Ile Ser Leu Pro Leu	465	470	475	480
Tyr Ser Ile Leu Ser Ala Leu Thr Ile Leu Gly Met Ile Met Ala Ser	485	490	495	40
Ala Phe Leu Phe Phe Asn Ile Lys Asn Arg Asn Gln Lys Leu Ile Lys	500	505	510	45
Met Ser Ser Pro Tyr Met Asn Asn Leu Ile Ile Leu Gly Gly Met Leu	515	520	525	50
Ser Tyr Ala Ser Ile Phe Leu Phe Gly Leu Asp Gly Ser Phe Val Ser	530	535	540	55
Glu Lys Thr Phe Glu Thr Leu Cys Thr Val Arg Thr Trp Ile Leu Thr	545	550	555	560
Val Gly Tyr Thr Thr Ala Phe Gly Ala Met Phe Ala Lys Thr Trp Arg	565	570	575	60
Val His Ala Ile Phe Lys Asn Val Lys Met Lys Lys Lys Ile Ile Lys	580	585	590	65
Asp Gln Lys Leu Leu Val Ile Val Gly Gly Met Leu Leu Ile Asp Leu	595	600	605	

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Cys Ile Leu Ile Cys Trp Gln Ala Val Asp Pro Leu Arg Arg Thr Val  
 610 615 620

5 Glu Arg Tyr Ser Met Glu Pro Asp Pro Ala Gly Arg Asp Ile Ser Ile  
 625 630 635 640

10 Arg Pro Leu Leu Glu His Cys Glu Asn Thr His Met Thr Ile Trp Leu  
 645 650 655

Gly Ile Val Tyr Ala Tyr Lys Gly Leu Leu Met Leu Phe Gly Cys Phe  
 660 665 670

15 Leu Ala Trp Glu Thr Arg Asn Val Ser Ile Pro Ala Leu Asn Asp Ser  
 675 680 685

20 Lys Tyr Ile Gly Met Ser Val Tyr Asn Val Gly Ile Met Cys Ile Ile  
 690 695 700

25 Gly Ala Ala Val Ser Phe Leu Thr Arg Asp Gln Pro Asn Val Gln Phe  
 705 710 715 720

Cys Ile Val Ala Leu Val Ile Ile Phe Cys Ser Thr Ile Thr Leu Cys  
 725 730 735

30 Leu Val Phe Val Pro Lys Leu Ile Thr Leu Arg Thr Asn Pro Asp Ala  
 740 745 750

35 Ala Thr Gln Asn Arg Arg Phe Gln Phe Thr Gln Asn Gln Lys Lys Glu  
 755 760 765

Asp Ser Lys Thr Ser Thr Ser Val Thr Ser Val Asn Gln Ala Ser Thr  
 770 775 780

40 Ser Arg Leu Glu Gly Leu Gln Ser Glu Asn His Arg Leu Arg Met Lys  
 785 790 795 800

45 Ile Thr Glu Leu Asp Lys Asp Leu Glu Glu Val Thr Met Gln Leu Gln  
 805 810 815

50 Asp Thr Pro Glu Lys Thr Thr Tyr Ile Lys Gln Asn His Tyr Gln Glu  
 820 825 830

Leu Asn Asp Ile Leu Ser Leu Gly Asn Phe Thr Glu Ser Thr Asp Gly  
 835 840 845

55 Gly Lys Ala Ile Leu Lys Asn His Leu Asp Gln Asn Pro Gln Leu Gln  
 850 855 860

60 Trp Asn Thr Thr Glu Pro Ser Arg Thr Cys Lys Asp Pro Ile Glu Asp  
 865 870 875 880

65 Ile Asn Ser Pro Glu His Ile Gln Arg Arg Leu Ser Leu Gln Leu Pro  
 885 890 895

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Ile Leu His His Ala Tyr Leu Pro Ser Ile Gly Gly Val Asp Ala Ser  
 900 905 910

Cys Val Ser Pro Cys Val Ser Pro Thr Ala Ser Pro Arg His Arg His  
 915 920 925

Val Pro Pro Ser Phe Arg Val Met Val Ser Gly Leu  
 930 935 940

(2) INFORMATION ZU SEQ ID NO: 3:

(i) SEQUENZ CHARAKTERISTIKA:

(A) LÄNGE: 2826 Basenpaare

(B) ART: Nukleinsäure

(C) STRANGFORM: Einzel

(D) TOPOLOGIE: linear

(ii) ART DES MOLEKÜLS: cDNS zu mRNS

(iii) HYPOTHETISCH: NEIN

(iii) ANTISENSE: NEIN

(vi) URSPRÜNGLICHE HERKUNFT:

(A) ORGANISMUS: Homo sapiens

(ix) MERKMALE:

(A) NAME/SCHLÜSSEL: CDS

(B) LÄNGE: 1..2826

(xi) SEQUENZBESCHREIBUNG: SEQ ID NO: 3:

ATG GCT TCC CCG CGG AGC TCC GGG CAG CCC GGG CCG CCG CCG CCG CCG  
 Met Ala Ser Pro Arg Ser Ser Gly Gln Pro Gly Pro Pro Pro Pro Pro  
 1 5 10 15

CCA CCG CCG CCC GCG CGC CTG CTA CTG CTA CTG CTG CTG CCG CTG CTG  
 Pro Pro Pro Pro Ala Arg Leu Leu Leu Leu Leu Leu Pro Leu Leu  
 20 25 30

CTG CCT CTG GCG CCC GGG GCC TGG GGC TGG GCG CCG GGC GCC CCC CCG  
 Leu Pro Leu Ala Pro Gly Ala Trp Gly Trp Ala Arg Gly Ala Pro Arg  
 35 40 45

CCG CCG CCC AGC AGC CCG CCG CTC TCC ATC ATG GGC CTC ATG CCG CTC  
 Pro Pro Pro Ser Ser Pro Pro Leu Ser Ile Met Gly Leu Met Pro Leu  
 50 55 60

ACC AAG GAG GTG GCC AAG GGC AGC ATC GGG CGC GGT GTG CTC CCC GCC  
 Thr Lys Glu Val Ala Lys Gly Ser Ile Gly Arg Gly Val Leu Pro Ala  
 65 70 75 80

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	GTG GAA CTG GCC ATC GAG CAG ATC CGC AAC GAG TCA CTC CTG CGC CCC	288
	Val Glu Leu Ala Ile Glu Gln Ile Arg Asn Glu Ser Leu Leu Arg Pro	
	85 90 95	
5	TAC TTC CTC GAC CTG CGG CTC TAT GAC ACG GAG TGC GAC AAC GCA AAA	336
	Tyr Phe Leu Asp Leu Arg Leu Tyr Asp Thr Glu Cys Asp Asn Ala Lys	
	100 105 110	
10	GGG TTG AAA GCC TTC TAC GAT GCA ATA AAA TAC GGG CCG AAC CAC TTG	384
	Gly Leu Lys Ala Phe Tyr Asp Ala Ile Lys Tyr Gly Pro Asn His Leu	
	115 120 125	
15	ATG GTG TTT GGA GGC GTC TGT CCA TCC GTC ACA TCC ATC ATT GCA GAG	432
	Met Val Phe Gly Gly Val Cys Pro Ser Val Thr Ser Ile Ile Ala Glu	
	130 135 140	
20	TCC CTC CAA GGC TGG AAT CTG GTG CAG CTT TCT TTT GCT GCA ACC ACG	480
	Ser Leu Gln Gly Trp Asn Leu Val Gln Leu Ser Phe Ala Ala Thr Thr	
	145 150 155 160	
25	CCT GTT CTA GCC GAT AAG AAA AAA TAC CCT TAT TTC TTT CGG ACC GTC	528
	Pro Val Leu Ala Asp Lys Lys Lys Tyr Pro Tyr Phe Phe Arg Thr Val	
	165 170 175	
30	CCA TCA GAC AAT GCG GTG AAT CCA GCC ATT CTG AAG TTG CTC AAG CAC	576
	Pro Ser Asp Asn Ala Val Asn Pro Ala Ile Leu Lys Leu Leu Lys His	
	180 185 190	
35	TAC CAG TGG AAG CGC GTG GGC ACG CTG ACG CAA GAC GTT CAG AGG TTC	624
	Tyr Gln Trp Lys Arg Val Gly Thr Leu Thr Gln Asp Val Gln Arg Phe	
	195 200 205	
40	TCT GAG GTG CGG AAT GAC CTG ACT GGA GTT CTG TAT GGC GAG GAC ATT	672
	Ser Glu Val Arg Asn Asp Leu Thr Gly Val Leu Tyr Gly Glu Asp Ile	
	210 215 220	
45	GAG ATT TCA GAC ACC GAG AGC TTC TCC AAC GAT CCC TGT ACC AGT GTC	720
	Glu Ile Ser Asp Thr Glu Ser Phe Ser Asn Asp Pro Cys Thr Ser Val	
	225 230 235 240	
50	AAA AAG CTG AAG GGC AAT GAT GTG CGG ATC ATC CTT GGC CAG TTT GAC	768
	Lys Lys Leu Lys Gly Asn Asp Val Arg Ile Ile Leu Gly Gln Phe Asp	
	245 250 255	
55	CAG AAT ATG GCA GCA AAA GTG TTC TGT TGT GCA TAC GAG GAG AAC ATG	816
	Gln Asn Met Ala Ala Lys Val Phe Cys Cys Ala Tyr Glu Glu Asn Met	
	260 265 270	
60	TAT GGT AGT AAA TAT CAG TGG ATC ATT CCG GGC TGG TAC GAG CCT TCT	864
	Tyr Gly Ser Lys Tyr Gln Trp Ile Ile Pro Gly Trp Tyr Glu Pro Ser	
	275 280 285	

65

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TGG TGG GAG CAG GTG CAC ACG GAA GCC AAC TCA TCC CGC TGC CTC CGG	912	
Trp Trp Glu Gln Val His Thr Glu Ala Asn Ser Ser Arg Cys Leu Arg		
290 295 300		5
AAG AAT CTG CTT GCT GCC ATG GAG GGC TAC ATT GGC GTG GAT TTC GAG	960	
Lys Asn Leu Leu Ala Ala Met Glu Gly Tyr Ile Gly Val Asp Phe Glu		
305 310 315 320		10
CCC CTG AGC TCC AAG CAG ATC AAG ACC ATC TCA GGA AAG ACT CCA CAG	1008	
Pro Leu Ser Ser Lys Gln Ile Lys Thr Ile Ser Gly Lys Thr Pro Gln		
325 330 335		15
CAG TAT GAG AGA GAG TAC AAC AAC AAG CGG TCA GGC GTG GGG CCC AGC	1056	
Gln Tyr Glu Arg Glu Tyr Asn Asn Lys Arg Ser Gly Val Gly Pro Ser		
340 345 350		20
AAG TTC CAC GGG TAC GCC TAC GAT GGC ATC TGG GTC ATC GCC AAG ACA	1104	
Lys Phe His Gly Tyr Ala Tyr Asp Gly Ile Trp Val Ile Ala Lys Thr		
355 360 365		25
CTG CAG AGG GCC ATG GAG ACA CTG CAT GCC AGC AGC CGG CAC CAG CGG	1152	
Leu Gln Arg Ala Met Glu Thr Leu His Ala Ser Ser Arg His Gln Arg		
370 375 380		30
ATC CAG GAC TTC AAC TAC ACG GAC CAC ACG CTG GGC AGG ATC ATC CTC	1200	
Ile Gln Asp Phe Asn Tyr Thr Asp His Thr Leu Gly Arg Ile Ile Leu		
385 390 395 400		35
AAT GCC ATG AAC GAG ACC AAC TTC TTC GGC GTC ACG GGT CAA GTT GTA	1248	
Asn Ala Met Asn Glu Thr Asn Phe Phe Gly Val Thr Gly Gln Val Val		
405 410 415		40
TTC CGG AAT GGG GAG AGA ATG GGG ACC ATT AAA TTT ACT CAA TTT CAA	1296	
Phe Arg Asn Gly Glu Arg Met Gly Thr Ile Lys Phe Thr Gln Phe Gln		
420 425 430		45
GAC AGC AGG GAG GTG AAG GTG GGA GAG TAC AAC GCT GTG GCC GAC ACA	1344	
Asp Ser Arg Glu Val Lys Val Gly Glu Tyr Asn Ala Val Ala Asp Thr		
435 440 445		50
CTG GAG ATC ATC AAT GAC ACC ATC AGG TTC CAA GGA TCC GAA CCA CCA	1392	
Leu Glu Ile Ile Asn Asp Thr Ile Arg Phe Gln Gly Ser Glu Pro Pro		
450 455 460		55
AAA GAC AAG ACC ATC ATC CTG GAG CAG CTG CGG AAG ATC TCC CTA CCT	1440	
Lys Asp Lys Thr Ile Ile Leu Glu Gln Leu Arg Lys Ile Ser Leu Pro		
465 470 475 480		60
CTC TAC AGC ATC CTC TCT GCC CTC ACC ATC CTC GGG ATG ATC ATG GCC	1488	
Leu Tyr Ser Ile Leu Ser Ala Leu Thr Ile Leu Gly Met Ile Met Ala		
485 490 495		65

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	AGT GCT TTT CTC TTC TTC AAC ATC AAG AAC CGG AAT CAG AAG CTC ATA	1536
	Ser Ala Phe Leu Phe Phe Asn Ile Lys Asn Arg Asn Gln Lys Leu Ile	
	500 505 510	
5	AAG ATG TCG AGT CCA TAC ATG AAC AAC CTT ATC ATC CTT GGA GGG ATG	1584
	Lys Met Ser Ser Pro Tyr Met Asn Asn Leu Ile Ile Leu Gly Gly Met	
	515 520 525	
10	CTC TCC TAT GCT TCC ATA TTT CTC TTT GGC CTT GAT GGA TCC TTT GTC	1632
	Leu Ser Tyr Ala Ser Ile Phe Leu Phe Gly Leu Asp Gly Ser Phe Val	
	530 535 540	
15	TCT GAA AAG ACC TTT GAA ACA CTT TGC ACC GTC AGG ACC TGG ATT CTC	1680
	Ser Glu Lys Thr Phe Glu Thr Leu Cys Thr Val Arg Thr Trp Ile Leu	
	545 550 555 560	
20	ACC GTG GGC TAC ACG ACC GCT TTT GGG GCC ATG TTT GCA AAG ACC TGG	1728
	Thr Val Gly Tyr Thr Thr Ala Phe Gly Ala Met Phe Ala Lys Thr Trp	
	565 570 575	
25	AGA GTC CAC GCC ATC TTC AAA AAT GTG AAA ATG AAG AAG AAG ATC ATC	1776
	Arg Val His Ala Ile Phe Lys Asn Val Lys Met Lys Lys Lys Ile Ile	
	580 585 590	
30	AAG GAC CAG AAA CTG CTT GTG ATC GTG GGG GGC ATG CTG CTG ATC GAC	1824
	Lys Asp Gln Lys Leu Leu Val Ile Val Gly Gly Met Leu Leu Ile Asp	
	595 600 605	
35	CTG TGT ATC CTG ATC TGC TGG CAG GCT GTG GAC CCC CTG CGA AGG ACA	1872
	Leu Cys Ile Leu Ile Cys Trp Gln Ala Val Asp Pro Leu Arg Arg Thr	
	610 615 620	
40	GTG GAG AAG TAC AGC ATG GAG CCG GAC CCA GCA GGA CGG GAT ATC TCC	1920
	Val Glu Lys Tyr Ser Met Glu Pro Asp Pro Ala Gly Arg Asp Ile Ser	
	625 630 635 640	
45	ATC CGC CCT CTC CTG GAG CAC TGT GAG AAC ACC CAT ATG ACC ATC TGG	1968
	Ile Arg Pro Leu Leu Glu His Cys Glu Asn Thr His Met Thr Ile Trp	
	645 650 655	
50	CTT GGC ATC GTC TAT GCC TAC AAG GGA CTT CTC ATG TTG TTC GGT TGT	2016
	Leu Gly Ile Val Tyr Ala Tyr Lys Gly Leu Leu Met Leu Phe Gly Cys	
	660 665 670	
55	TTC TTA GCT TGG GAG ACC CGC AAC GTC AGC ATC CCC GCA CTC AAC GAC	2064
	Phe Leu Ala Trp Glu Thr Arg Asn Val Ser Ile Pro Ala Leu Asn Asp	
	675 680 685	
60	AGC AAG TAC ATC GGG ATG AGT GTC TAC AAC GTG GGG ATC ATG TGC ATC	2112
	Ser Lys Tyr Ile Gly Met Ser Val Tyr Asn Val Gly Ile Met Cys Ile	
	690 695 700	

65

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ATC GGG GCC GCT GTC TCC TTC CTG ACC CGG GAC CAG CCC AAT GTG CAG Ile Gly Ala Ala Val Ser Phe Leu Thr Arg Asp Gln Pro Asn Val Gln 705 710 715 720	2160	5
TTC TGC ATC GTG GCT CTG GTC ATC ATC TTC TGC AGC ACC ATC ACC CTC Phe Cys Ile Val Ala Leu Val Ile Ile Phe Cys Ser Thr Ile Thr Leu 725 730 735	2208	10
TGC CTG GTA TTC GTG CCG AAG CTC ATC ACC CTG AGA ACA AAC CCA GAT Cys Leu Val Phe Val Pro Lys Leu Ile Thr Leu Arg Thr Asn Pro Asp 740 745 750	2256	15
GCA GCA ACG CAG AAC AGG CGA TTC CAG TTC ACT CAG AAT CAG AAG AAA Ala Ala Thr Gln Asn Arg Arg Phe Gln Phe Thr Gln Asn Gln Lys Lys 755 760 765	2304	20
GAA GAT TCT AAA ACG TCC ACC TCG GTC ACC AGT GTG AAC CAA GCC AGC Glu Asp Ser Lys Thr Ser Thr Ser Val Thr Ser Val Asn Gln Ala Ser 770 775 780	2352	25
ACA TCC CGC CTG GAG GGC CTA CAG TCA GAA AAC CAT CGC CTG CGA ATG Thr Ser Arg Leu Glu Gly Leu Gln Ser Glu Asn His Arg Leu Arg Met 785 790 795 800	2400	30
AAG ATC ACA GAG CTG GAT AAA GAC TTG GAA GAG GTC ACC ATG CAG CTG Lys Ile Thr Glu Leu Asp Lys Asp Leu Glu Glu Val Thr Met Gln Leu 805 810 815	2448	35
CAG GAC ACA CCA GAA AAG ACC ACC TAC ATT AAA CAG AAC CAC TAC CAA Gln Asp Thr Pro Glu Lys Thr Thr Tyr Ile Lys Gln Asn His Tyr Gln 820 825 830	2496	40
GAG CTC AAT GAC ATC CTC AAC CTG GGA AAC TTC ACT GAG AGC ACA GAT Glu Leu Asn Asp Ile Leu Asn Leu Gly Asn Phe Thr Glu Ser Thr Asp 835 840 845	2544	45
GGA GGA AAG GCC ATT TTA AAA AAT CAC CTC GAT CAA AAT CCC CAG CTA Gly Gly Lys Ala Ile Leu Lys Asn His Leu Asp Gln Asn Pro Gln Leu 850 855 860	2592	50
CAG TGG AAC ACA ACA GAG CCC TCT CGA ACA TGC AAA GAT CCT ATA GAA Gln Trp Asn Thr Thr Glu Pro Ser Arg Thr Cys Lys Asp Pro Ile Glu 865 870 875 880	2640	55
GAT ATA AAC TCT CCA GAA CAC ATC CAG CGT CGG CTG TCC CTC CAG CTC Asp Ile Asn Ser Pro Glu His Ile Gln Arg Arg Leu Ser Leu Gln Leu 885 890 895	2688	60
CCC ATC CTC CAC CAC GCC TAC CTC CCA TCC ATC GGA GGC GTG GAC GCC Pro Ile Leu His His Ala Tyr Leu Pro Ser Ile Gly Gly Val Asp Ala 900 905 910	2736	65



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AGC TGT GTC AGC CCC TGC GTC AGC CCC ACC GCC AGC CCC CGC CAC AGA  
Ser Cys Val Ser Pro Cys Val Ser Pro Thr Ala Ser Pro Arg His Arg  
915 920 925

2784

CAT GTG CCA CCC TCC TTC CGA GTC ATG GTC TCG GGC CTG TAA  
His Val Pro Pro Ser Phe Arg Val Met Val Ser Gly Leu  
930 935 940

2826

(2) INFORMATION ZU SEQ ID NO: 4:

(i) SEQUENZ CHARAKTERISTIKA:

(A) LÄNGE: 941 Aminosäuren

(B) ART: Aminosäure

(D) TOPOLOGIE: linear

(ii) ART DES MOLEKÜLS: Protein

(xi) SEQUENZBESCHREIBUNG: SEQ ID NO: 4:

Met Ala Ser Pro Arg Ser Ser Gly Gln Pro Gly Pro Pro Pro Pro Pro  
1 5 10 15

Pro Pro Pro Pro Ala Arg Leu Leu Leu Leu Leu Leu Leu Pro Leu Leu  
20 25 30

Leu Pro Leu Ala Pro Gly Ala Trp Gly Trp Ala Arg Gly Ala Pro Arg  
35 40 45

Pro Pro Pro Ser Ser Pro Pro Leu Ser Ile Met Gly Leu Met Pro Leu  
50 55 60

Thr Lys Glu Val Ala Lys Gly Ser Ile Gly Arg Gly Val Leu Pro Ala  
65 70 75 80

Val Glu Leu Ala Ile Glu Gln Ile Arg Asn Glu Ser Leu Leu Arg Pro  
85 90 95

Tyr Phe Leu Asp Leu Arg Leu Tyr Asp Thr Glu Cys Asp Asn Ala Lys  
100 105 110

Gly Leu Lys Ala Phe Tyr Asp Ala Ile Lys Tyr Gly Pro Asn His Leu  
115 120 125

Met Val Phe Gly Gly Val Cys Pro Ser Val Thr Ser Ile Ile Ala Glu  
130 135 140

Ser Leu Gln Gly Trp Asn Leu Val Gln Leu Ser Phe Ala Ala Thr Thr  
145 150 155 160

Pro Val Leu Ala Asp Lys Lys Lys Tyr Pro Tyr Phe Phe Arg Thr Val  
165 170 175

Pro Ser Asp Asn Ala Val Asn Pro Ala Ile Leu Lys Leu Leu Lys His  
180 185 190

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Tyr	Gln	Trp	Lys	Arg	Val	Gly	Thr	Leu	Thr	Gln	Asp	Val	Gln	Arg	Phe		
195							200					205					
Ser	Glu	Val	Arg	Asn	Asp	Leu	Thr	Gly	Val	Leu	Tyr	Gly	Glu	Asp	Ile		5
210						215					220						
Glu	Ile	Ser	Asp	Thr	Glu	Ser	Phe	Ser	Asn	Asp	Pro	Cys	Thr	Ser	Val		10
225					230					235					240		
Lys	Lys	Leu	Lys	Gly	Asn	Asp	Val	Arg	Ile	Ile	Leu	Gly	Gln	Phe	Asp		15
				245					250					255			
Gln	Asn	Met	Ala	Ala	Lys	Val	Phe	Cys	Cys	Ala	Tyr	Glu	Glu	Asn	Met		
			260					265					270				
Tyr	Gly	Ser	Lys	Tyr	Gln	Trp	Ile	Ile	Pro	Gly	Trp	Tyr	Glu	Pro	Ser		20
	275					280						285					
Trp	Trp	Glu	Gln	Val	His	Thr	Glu	Ala	Asn	Ser	Ser	Arg	Cys	Leu	Arg		25
	290					295					300						
Lys	Asn	Leu	Leu	Ala	Ala	Met	Glu	Gly	Tyr	Ile	Gly	Val	Asp	Phe	Glu		
305					310					315					320		
Pro	Leu	Ser	Ser	Lys	Gln	Ile	Lys	Thr	Ile	Ser	Gly	Lys	Thr	Pro	Gln		30
				325					330					335			
Gln	Tyr	Glu	Arg	Glu	Tyr	Asn	Asn	Lys	Arg	Ser	Gly	Val	Gly	Pro	Ser		35
		340						345					350				
Lys	Phe	His	Gly	Tyr	Ala	Tyr	Asp	Gly	Ile	Trp	Val	Ile	Ala	Lys	Thr		40
	355						360					365					
Leu	Gln	Arg	Ala	Met	Glu	Thr	Leu	His	Ala	Ser	Ser	Arg	His	Gln	Arg		
370						375					380						
Ile	Gln	Asp	Phe	Asn	Tyr	Thr	Asp	His	Thr	Leu	Gly	Arg	Ile	Ile	Leu		45
385				390						395					400		
Asn	Ala	Met	Asn	Glu	Thr	Asn	Phe	Phe	Gly	Val	Thr	Gly	Gln	Val	Val		50
				405					410					415			
Phe	Arg	Asn	Gly	Glu	Arg	Met	Gly	Thr	Ile	Lys	Phe	Thr	Gln	Phe	Gln		
		420					425						430				
Asp	Ser	Arg	Glu	Val	Lys	Val	Gly	Glu	Tyr	Asn	Ala	Val	Ala	Asp	Thr		55
		435					440					445					
Leu	Glu	Ile	Ile	Asn	Asp	Thr	Ile	Arg	Phe	Gln	Gly	Ser	Glu	Pro	Pro		60
	450				455						460						
Lys	Asp	Lys	Thr	Ile	Ile	Leu	Glu	Gln	Leu	Arg	Lys	Ile	Ser	Leu	Pro		65
465				470						475					480		

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Leu Tyr Ser Ile Leu Ser Ala Leu Thr Ile Leu Gly Met Ile Met Ala  
 485 490 495  
 5 Ser Ala Phe Leu Phe Phe Asn Ile Lys Asn Arg Asn Gln Lys Leu Ile  
 500 505 510  
 10 Lys Met Ser Ser Pro Tyr Met Asn Asn Leu Ile Ile Leu Gly Gly Met  
 515 520 525  
 Leu Ser Tyr Ala Ser Ile Phe Leu Phe Gly Leu Asp Gly Ser Phe Val  
 530 535 540  
 15 Ser Glu Lys Thr Phe Glu Thr Leu Cys Thr Val Arg Thr Trp Ile Leu  
 545 550 555 560  
 20 Thr Val Gly Tyr Thr Thr Ala Phe Gly Ala Met Phe Ala Lys Thr Trp  
 565 570 575  
 Arg Val His Ala Ile Phe Lys Asn Val Lys Met Lys Lys Lys Ile Ile  
 25 580 585 590  
 Lys Asp Gln Lys Leu Leu Val Ile Val Gly Gly Met Leu Leu Ile Asp  
 595 600 605  
 30 Leu Cys Ile Leu Ile Cys Trp Gln Ala Val Asp Pro Leu Arg Arg Thr  
 610 615 620  
 35 Val Glu Lys Tyr Ser Met Glu Pro Asp Pro Ala Gly Arg Asp Ile Ser  
 625 630 635 640  
 Ile Arg Pro Leu Leu Glu His Cys Glu Asn Thr His Met Thr Ile Trp  
 40 645 650 655  
 Leu Gly Ile Val Tyr Ala Tyr Lys Gly Leu Leu Met Leu Phe Gly Cys  
 660 665 670  
 45 Phe Leu Ala Trp Glu Thr Arg Asn Val Ser Ile Pro Ala Leu Asn Asp  
 675 680 685  
 50 Ser Lys Tyr Ile Gly Met Ser Val Tyr Asn Val Gly Ile Met Cys Ile  
 690 695 700  
 Ile Gly Ala Ala Val Ser Phe Leu Thr Arg Asp Gln Pro Asn Val Gln  
 705 710 715 720  
 55 Phe Cys Ile Val Ala Leu Val Ile Ile Phe Cys Ser Thr Ile Thr Leu  
 725 730 735  
 60 Cys Leu Val Phe Val Pro Lys Leu Ile Thr Leu Arg Thr Asn Pro Asp  
 740 745 750  
 Ala Ala Thr Gln Asn Arg Arg Phe Gln Phe Thr Gln Asn Gln Lys Lys  
 65 755 760 765

Glu	Asp	Ser	Lys	Thr	Ser	Thr	Ser	Val	Thr	Ser	Val	Asn	Gln	Ala	Ser		
770						775					780						
Thr	Ser	Arg	Leu	Glu	Gly	Leu	Gln	Ser	Glu	Asn	His	Arg	Leu	Arg	Met		5
785					790				795						800		
Lys	Ile	Thr	Glu	Leu	Asp	Lys	Asp	Leu	Glu	Glu	Val	Thr	Met	Gln	Leu		10
				805					810					815			
Gln	Asp	Thr	Pro	Glu	Lys	Thr	Thr	Tyr	Ile	Lys	Gln	Asn	His	Tyr	Gln		15
				820				825					830				
Glu	Leu	Asn	Asp	Ile	Leu	Asn	Leu	Gly	Asn	Phe	Thr	Glu	Ser	Thr	Asp		
				835			840					845					
Gly	Gly	Lys	Ala	Ile	Leu	Lys	Asn	His	Leu	Asp	Gln	Asn	Pro	Gln	Leu		20
						855					860						
Gln	Trp	Asn	Thr	Thr	Glu	Pro	Ser	Arg	Thr	Cys	Lys	Asp	Pro	Ile	Glu		25
865					870					875					880		
Asp	Ile	Asn	Ser	Pro	Glu	His	Ile	Gln	Arg	Leu	Ser	Leu	Gln	Leu			
				885					890					895			30
Pro	Ile	Leu	His	His	Ala	Tyr	Leu	Pro	Ser	Ile	Gly	Gly	Val	Asp	Ala		
				900				905					910				
Ser	Cys	Val	Ser	Pro	Cys	Val	Ser	Pro	Thr	Ala	Ser	Pro	Arg	His	Arg		35
		915					920					925					
His	Val	Pro	Pro	Ser	Phe	Arg	Val	Met	Val	Ser	Gly	Leu					40
		930				935					940						

## Patentansprüche

1. Proteinheteromer, enthaltend mindestens ein GABA<sub>B</sub>-Rezeptorprotein und mindestens ein Protein mit der in SEQ ID NO: 2 oder SEQ ID NO: 4 dargestellte Aminosäuresequenz oder eine daraus durch Substitution, Inversion, Insertion oder Deletion von einem oder mehreren Aminosäureresten erhaltliche Sequenz, wobei wenigstens noch eine der wesentlichen biologischen Eigenschaften des in SEQ ID NO: 2 oder SEQ ID NO: 4 dargestellten Proteins oder des Proteinheteromer erhalten bleibt. 45
2. Isoliertes Protein, enthaltend die in SEQ ID NO: 2 oder SEQ ID NO: 4 dargestellte Aminosäuresequenz oder eine daraus durch Substitution, Inversion, Insertion oder Deletion von einem oder mehreren Aminosäureresten erhaltliche Sequenz, wobei wenigstens noch eine der wesentlichen biologischen Eigenschaften des in SEQ ID NO: 2 oder SEQ ID NO: 4 dargestellten Proteins erhalten bleibt. 50
3. Nukleinsäuresequenz codierend für ein Protein gemäß Anspruch 2.
4. Nukleinsäuresequenz nach Anspruch 3, dadurch gekennzeichnet, daß sie für ein Protein codiert, das wenigstens 60% Identität mit der in SEQ ID NO: 2 oder SEQ ID NO: 4 dargestellten Sequenz hat. 55
5. Nukleinsäuresequenz nach Anspruch 3, dadurch gekennzeichnet, daß sie die in SEQ ID NO: 1 oder SEQ ID NO: 3 dargestellte Sequenz enthält.
6. Rekombinantes Nukleinsäurekonstrukt, enthaltend eine Nukleinsäuresequenz gemäß Anspruch 3 oder eine Nukleinsäuresequenz gemäß Anspruch 3 und eine Sequenz, die für ein GABA<sub>B</sub>-Rezeptorprotein kodiert, funktionell verknüpft mit mindestens einem genetischen Regulationselement. 60
7. Wirtsorganismus, transformiert mit einer Nukleinsäuresequenz gemäß Anspruch 3 oder einem rekombinanten Nukleinsäurekonstrukt gemäß Anspruch 6 oder mit einer Nukleinsäuresequenz gemäß Anspruch 3 oder einem rekombinanten Nukleinsäurekonstrukt gemäß Anspruch 6 zusammen mit einer Sequenz, die für ein GABA<sub>B</sub>-Rezeptorprotein kodiert.
8. Wirtsorganismus, transformiert mit einem rekombinanten Nukleinsäurekonstrukt gemäß Anspruch 6. 65
9. Transgene Tiere enthaltend eine funktionelle oder nicht funktionelle Nukleinsäuresequenz gemäß den Ansprüchen 3 bis 5 oder ein funktionelles oder nicht funktionelles Nukleinsäurekonstrukt nach Anspruch 6.
10. Transgene Tiere, in dessen Keimzellen oder der Gesamtheit oder einem Teil der somatischen Zellen, oder in

dessen Keimzellen und der Gesamtheit oder einem Teil der somatischen Zellen die Nukleotidsequenz gemäß Anspruch 3 durch gentechnische Verfahren verändert oder durch Einfügen von DNA-Elementen unterbrochen wurde.

11. Verwendung einer Nukleinsäuresequenz gemäß Anspruch 3, eines Nukleinsäurekonstrukts gemäß Anspruch 6, eines Proteinheteromeren gemäß Anspruch 1 oder Proteins gemäß Anspruch 2 zur Identifizierung von Proteinen, die zu einem Proteinheteromeren gemäß Anspruch 1 oder einem Protein gemäß Anspruch 2 spezifische Bindungsaffinitäten aufweisen, oder zur Identifizierung von Nukleinsäuren, die für Proteine kodieren, die zu einem Proteinheteromeren gemäß Anspruch 1 oder einem Protein gemäß Anspruch 2 spezifische Bindungsaffinitäten aufweisen.

12. Verwendung des two-hybrid Systems oder biochemischer Verfahren zur Identifizierung der Interaktionsdomänen von metabotropen Rezeptoren und Verwendung zur pharmakotherapeutischen Intervention.

13. Verwendung der aus einer Strukturaufklärung eines Proteinheteromeren gemäß Anspruch 1 oder eines Proteins gemäß Anspruch 2 resultierenden Information zum gezielten Auffinden oder zur gezielten Herstellung von Substanzen mit spezifischer Bindungsaktivität zu einem Proteinheteromeren gemäß Anspruch 1 oder einem Protein gemäß Anspruch 2.

14. Verwendung eines Proteinheteromeren gemäß Anspruch 1 oder eines Proteins gemäß Anspruch 2 oder Peptidfragmenten davon als Antigen zur Erzeugung von spezifischen mono- oder polyklonalen Antikörpern oder Antikörpergemischen gerichtet gegen Proteine gemäß Anspruch 1 oder 2.

15. Mono- oder polyklonale Antikörper oder Antikörpergemische, die spezifisch Proteine nach Anspruch 1 oder 2 erkennen.

16. Verwendung einer Nukleinsäuresequenz gemäß Anspruch 3 oder eines Fragmentes davon zur Isolierung einer genomischen Sequenz über Homologiescreening.

17. Verwendung einer Nukleinsäuresequenz gemäß Anspruch 3 als Marker für humane Erbkrankheiten.

18. Verwendung einer Nukleinsäuresequenz nach Anspruch 3 oder Teilen davon zur Gentherapie.

19. Verwendung einer zu der Nukleinsäuresequenz gemäß Anspruch 3 oder zu Teilen von ihr komplementären Nukleinsäuresequenz zur Gentherapie.

20. Verfahren zum Auffinden von Substanzen mit spezifischer Bindungsaffinität zu einem Protein nach Anspruch 1 oder 2, das folgende Schritte umfaßt:

a) Inkubation des Proteins gemäß Anspruch 1 oder 2 mit der zu testenden Substanz.

b) Detektion der Bindung der zu testenden Substanz an das Protein.

21. Verfahren nach Anspruch 20, dadurch gekennzeichnet, daß die Detektion der Bindung durch Messen der Antagonisierung oder Agonisierung der GABA<sub>A</sub>-Rezeptor-Aktivität erfolgt.

22. Verfahren nach Anspruch 20, dadurch gekennzeichnet, daß die Detektion der Bindung von Substanzen an ein Protein gemäß Anspruch 1 durch Messen einer physiologischen Wirkung, wie z. B. einer Änderung der Calcium-, cAMP-, IP<sub>3</sub>-Konzentration oder des Membranpotentials, erfolgt.

23. Verfahren zum qualitativen oder quantitativen Nachweis einer Nukleinsäure gemäß Anspruch 3 in einer biologischen Probe, das einen oder mehrere der folgenden Schritte umfaßt:

a) Inkubation einer biologischen Probe mit einer bekannten Menge an Nukleinsäure gemäß Anspruch 3 oder einer bekannten Menge an Oligonukleotiden, die als Primer für eine Amplifikation der Nukleinsäure gemäß Anspruch 3 geeignet sind oder Mischungen davon,

b) Nachweis der Nukleinsäure gemäß Anspruch 3 durch spezifische Hybridisierung oder PCR-Amplifikation,

c) Vergleich der Menge an hybridisierender Nukleinsäure gemäß Anspruch 3 oder an durch PCR Amplifikation gewonnener Nukleinsäure gemäß Anspruch 3 mit einem Standard.

24. Verfahren zum qualitativen und quantitativen Nachweis eines Proteins gemäß Anspruch 1 oder 2 in einer biologischen Probe, das einen oder mehrere der folgenden Schritte umfaßt:

a) Inkubation einer biologischen Probe mit einem Antikörper gemäß Anspruch 15, der spezifisch gegen Proteine gemäß Anspruch 1 oder 2 gerichtet ist,

b) Nachweis des Antikörper/Antigenkomplexes,

c) Vergleich der Mengen des Antikörper/Antigenkomplexes mit einem Mengenstandard.

25. Verfahren zum Auffinden von Substanzen, die spezifisch an ein Protein mit einer Aminosäuresequenz gemäß Anspruch 2 binden, das einen oder mehrere der folgenden Schritte umfaßt:

a) Expression des Proteins in eukaryotischen oder prokaryotischen Zellen.

b) Inkubation des Proteins mit den zu testenden Substanzen.

c) Nachweis der Bindung einer Substanz an den Rezeptor bzw. eines Effektes auf die Rezeptorfunktion.

26. Verfahren zum Auffinden von Substanzen, die spezifisch an ein Protein mit einer Aminosäuresequenz gemäß Anspruch 2 bzw. an eine Nukleinsäuresequenz gemäß Anspruch 3 binden und dadurch hemmende oder aktivierende funktionelle Effekte auf die GABAerge Signalweiterleitung in zentralnervösen Neuronen hervorrufen.

27. Verfahren zum Auffinden von Substanzen, die die Interaktion von Proteinen mit Aminosäuresequenzen gemäß Anspruch 2 mit anderen metabotropen Rezeptoren hemmen oder verstärken.

28. Verfahren zum Auffinden von Substanzen, die die Interaktion von Liganden mit dem Proteinheteromer gemäß Anspruch 1 oder Proteinen mit Aminosäuresequenzen gemäß Anspruch 2 hemmen oder verstärken.

29. Verfahren zum Auffinden von Substanzen, die die Interaktion von Proteinen mit Aminosäuresequenzen gemäß Anspruch 2 mit G-Proteinen oder anderen Signaltransduktionsmolekülen hemmen oder verstärken.

30. Verfahren zur qualitativen und quantitativen Bestimmung von Proteinen gemäß Anspruch 2 unter Benutzung von spezifischen Agonisten oder Antagonisten.

31. Verfahren zur Quantifizierung der Proteinaktivität eines Proteins gemäß Anspruch 2.

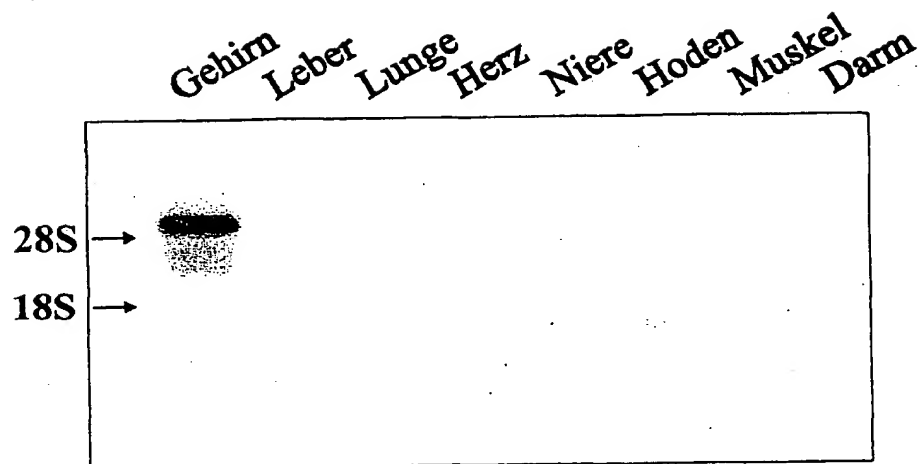
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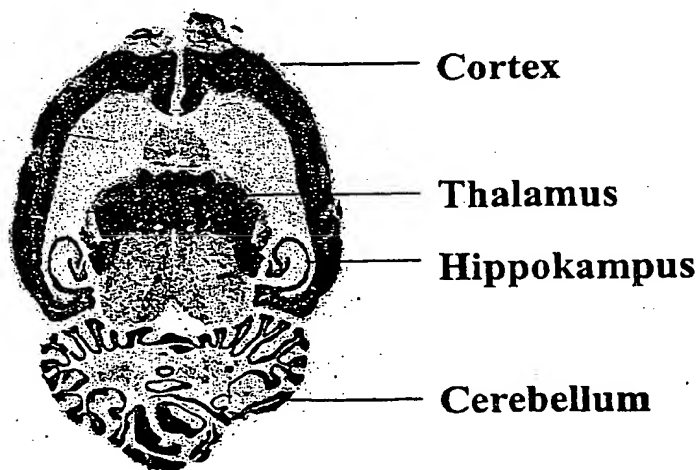
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Figur 1

A)



B)



GBR1A 1 MLLLLLVLFLRPLGAGGAQTPNATSEGCQIHPPEGGIRYGLTRDQVKAINFLPVDDY  
GBR1B 1 M.....CPGCPCTPVG.....MP.....LPL..  
SEQ2 1 MA.....SPSSGQRPPE.....PPPPPPARL.....LPL..  
61 EIEYVCRGEREWGPKVRKCLANGSWTMDTPSRCVURICSKSYLTENCKVFTTGGDLPA  
GBR1A 17 .....LLVMAAGVAPVWASHSPH  
GBR1B 27 .....LISLLW...LAPGANG.....  
SEQ2 121 LDGARVEFRCDPFDHLVSSRSVCSQGMSTPKPHCQVNRTPH  
SERRAVYIGALFFPMSSGG  
GBR1A 35 L.....PPHPRVPPHPS  
GBR1B 41 .....MA.....RGAPRPFPSSPPPSIMGLMPITKE  
SEQ2 181/65 WPGG...QACQPAVEMALIEDVNSRRDILPDYELKLIHHDISKDPCQATKYLYELLYNDPI  
GBR1A/B 67 VAKCSIGRCVLPVAVELATEQERNES...LRPYFLDLRLYDTECDNAKGLKAFYDAIKYGEN  
SEQ2 238/122 KTELMPC.GSSVSIVVAEAAARMNLIVLSVCS SSPALSNRQRPPIFFRTHPSATLHNETR  
GBR1A/B 126 HLMVFGVVCPSVSESIIAESLOGMNLQLSNAATPVLDKQKQVYFFRTVPSDNAVNPAL  
SEQ2 297/181 VKLFEKMGWKHATITQOTTEVFISTLDDLEERVKEAGIEETFRQSFSDPAVPVKNLKRQ  
186 KKLKHERNRNGITQDVQRFSEVRNDLTGVLYGEDIELSDTESFSNDPCTSVKKLKGN  
GBR1A/B 357/241 DARILVCLFYETEARKVFCVYKERIEGKKVVMFIIIGWYADNMFKTYDPSIN...CTVEE  
246 DRIILICQFDQNMMAKVFCACAEESMEGSKYQWIIIPGWYEPANWEQVHVHEANSSRCLRRS  
GBR1A/B 414/298 MTEAVECHITTTIIVMNPANTRISINMTSQERVEKLTKRILKRHPEETGCFQEAPLAYDAL  
306 ELAAMEGYIGVDFEPLSSKOIKIISGKTPOQXEREYNS...KRSGVGPSKPHG..YAYDEI  
SEQ2 474/358 MATAALANKTSGG..GGRSGVRLEDNFYNNQITDDQIYRAMNSSSEGVSGHVVDASGS  
362 MVIAKTORAMETLHASRRHQRTQDENYTDHTLGKILINANNERNFFGVIGQVFR.NGE  
GBR1A/B 532/416 RMAWTLIEQLGGSYKIGYDSTKDDLSW.SKTDKMTIGGSPPADOTVTKTFFFSQKL  
421 RMGTIKFTQFQDSREVKVGEYNAVADTLEIINDTIFRQCSPPKDKTILEQLKSLPL

Figur 2



cells, and new and useful methods for cultured growth of such host cells capable of expression of the polypeptide and related products.

[0132] Vectors which comprise the isolated nucleic acid molecule described hereinabove also are provided. Suitable vectors comprise, but are not limited to, a plasmid or a virus. These vectors may be transformed into a suitable host cell to form a host cell expression system for the production of a GABA<sub>B</sub>R2 polypeptide. Suitable host cells include, for example, neuronal cells such as the glial cell line C6, a *Xenopus* cell such as an oocyte or melanophore cell, as well as numerous mammalian cells and non-neuronal cells.

[0133] This invention further provides for any vector or plasmid which comprises modified untranslated sequences, which are beneficial for expression in desired host cells or for use in binding or functional assays. For example, a vector or plasmid with untranslated sequences of varying lengths may express differing amounts of the polypeptide depending upon the host cell used. In an embodiment, the vector or plasmid comprises the coding sequence of the polypeptide and the regulatory elements necessary for expression in the host cell.

[0134] As used herein, the phrase "specifically hybridizing" means the ability of a nucleic acid molecule to recognize a nucleic acid sequence complementary to its own and to form double-helical segments through hydrogen bonding between complementary base pairs. The term "complementary" is used in its usual sense in the art, i.e., G and C are complementary and A is complementary to T (or U in RNA), such that two strands of nucleic acid are "complementary" only if every base matches the opposing base exactly.

[0135] This invention is directed to a purified GABA<sub>B</sub>R2 protein.

[0136] This invention is directed to a vector comprising a above-identified nucleic acid.

[0137] In one embodiment, the vector is adapted for expression in a bacterial cell which comprises the regulatory elements necessary for expression of the nucleic acid in the bacterial cell operatively linked to the nucleic acid encoding a GABA<sub>B</sub>R2 polypeptide so as to permit expression thereof.

[0138] In another embodiment, the vector is adapted for expression in an amphibian cell which comprises the regulatory elements necessary for expression of the nucleic acid in the amphibian cell operatively linked to the nucleic acid encoding a GABA<sub>B</sub>R2 polypeptide so as to permit expression thereof.

[0139] In another embodiment, the vector is adapted for expression in a yeast cell which comprises the regulatory elements necessary for expression of the nucleic acid in the yeast cell operatively linked to the nucleic acid encoding a GABA<sub>B</sub>R2 polypeptide so as to permit expression thereof.

[0140] In another embodiment, the vector is adapted for expression in an insect cell which comprises the regulatory elements necessary for expression of the nucleic acid in the insect cell operatively linked to the nucleic acid encoding the GABA<sub>B</sub>R2 polypeptide so as to permit expression thereof.

[0141] In one embodiment, the vector is a baculovirus.

[0142] In another embodiment, the vector is adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the nucleic acid in the mammalian cell operatively linked to the nucleic acid encoding a GABA<sub>B</sub>R2 polypeptide so as to permit expression thereof.

[0143] In one embodiment, the vector is a plasmid.

[0144] In a further embodiment, the plasmid is designated BO-55 (ATCC Accession No. 209104).

[0145] In a further embodiment, the plasmid is designated pEXJT3T7-hGABAB2 (ATCC Accession No. 203515).

[0146] This invention provides a plasmid designated pEXJT3T7-hGABAB2 (ATCC Accession No. 203515) which comprises the regulatory elements necessary for expression of DNA in a mammalian cell operatively linked to DNA encoding the human polypeptide so as to permit expression thereof.

[0147] This plasmid (pEXJT3T7-hGABAB2) was deposited on Dec. 9, 1998, with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, Va. 20110-2209, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and was accorded ATCC Accession No. 203515.

[0148] This invention provides a plasmid designated BO-55 (ATCC Accession No. 209104) which comprises the regulatory elements necessary for expression of DNA in a mammalian cell operatively linked to DNA encoding the rat polypeptide so as to permit expression thereof.

[0149] This plasmid (BO-55) was deposited on Jun. 10, 1997, with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Md. 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and was accorded ATCC Accession No. 209104.

[0150] Nucleic acid probe technology is well known to those skilled in the art who will readily appreciate that such probes may vary greatly in length and may be labeled with a detectable label, such as a radioisotope or fluorescent dye, to facilitate detection of the probe. DNA probe molecules may be produced by insertion of a DNA molecule which encodes the polypeptides of this invention into suitable vectors, such as plasmids or bacteriophages, followed by transforming into suitable bacterial host cells, replication in the transformed bacterial host cells and harvesting of the DNA probes, using methods well known in the art. Alternatively, probes may be generated chemically from DNA synthesizers.

[0151] RNA probes may be generated by inserting the DNA molecule which encodes the polypeptides of this invention downstream of a bacteriophage promoter such as T3, T7 or SP6. Large amounts of RNA probe may be produced by incubating the labeled nucleotides with the linearized fragment where it contains an upstream promoter in the presence of the appropriate RNA polymerase.

[0152] This invention is directed to a method of detecting a nucleic acid encoding a GABA<sub>B</sub>R2 polypeptide, which comprises contacting the nucleic acid with a probe compris-

ing at least 15 nucleotides, which probe specifically hybridizes with the nucleic acid encoding the GABA<sub>B</sub>R2 polypeptide, wherein the probe has a unique sequence, which sequence is present within one of the two strands of the nucleic acid encoding the GABA<sub>B</sub>R2 polypeptide contained in plasmid BO-55, and detecting hybridization of the probe to the nucleic acid.

[0153] This invention is directed to a method of detecting a nucleic acid encoding a GABA<sub>B</sub>R2 polypeptide, which comprises contacting the nucleic acid with a probe comprising at least 15 nucleotides, which probe specifically hybridizes with the nucleic acid encoding the GABA<sub>B</sub>R2 polypeptide, wherein the probe has a unique sequence, which sequence is present within (a) the nucleic acid sequence shown in FIGS. 22A-22D (SEQ ID NO:46) or (b) the reverse complement to the nucleic acid sequence shown in FIGS. 22A-22D (SEQ ID NO:46), and detecting hybridization of the probe to the nucleic acid.

[0154] This invention is directed to a method of detecting a nucleic acid encoding a GABA<sub>B</sub>R2 polypeptide, which comprises contacting the nucleic acid with a probe comprising at least 15 nucleotides, which probe specifically hybridizes with the nucleic acid encoding the GABA<sub>B</sub>R2 polypeptide, wherein the probe has a unique sequence, which sequence is present within one of the two strands of the nucleic acid encoding the GABA<sub>B</sub>R2 polypeptide contained in plasmid pEXJT3T7-hGABAB2 and detecting hybridization of the probe to the nucleic acid.

[0155] This invention is directed to a method of detecting a nucleic acid encoding a GABA<sub>B</sub>R2 polypeptide, which comprises contacting the nucleic acid with a probe comprising at least 15 nucleotides, which probe specifically hybridizes with the nucleic acid encoding the GABA<sub>B</sub>R2 polypeptide, wherein the probe has a unique sequence, which sequence is present within (a) the nucleic acid sequence shown in FIGS. 3A-3D (SEQ ID NO:3) or (b) the reverse complement to the nucleic acid sequence shown in FIGS. 3A-3D (SEQ ID NO:3), and detecting hybridization of the probe to the nucleic acid.

[0156] In one embodiment, the nucleic acid is DNA.

[0157] In another embodiment, the nucleic acid is RNA.

[0158] In one embodiment, the probe comprises at least 15 nucleotides complementary to a unique segment of the sequence of the nucleic acid molecule encoding the GABA<sub>B</sub>R2 polypeptide.

[0159] This invention is directed to a method of detecting a nucleic acid encoding a GABA<sub>B</sub>R2 polypeptide, which comprises contacting the nucleic acid with a probe comprising a nucleic acid of at least 15 nucleotides which is complementary to the antisense sequence of a unique segment of the sequence of the nucleic acid encoding the GABA<sub>B</sub>R2 polypeptide, and detecting hybridization of the probe to the nucleic acid.

[0160] This invention is directed to a method of inhibiting translation of mRNA encoding a GABA<sub>B</sub>R2 polypeptide which comprises contacting such mRNA with an antisense oligonucleotide having a sequence capable of specifically hybridizing to the above-identified mRNA, so as to prevent translation of the mRNA.

[0161] This invention is directed to a method of inhibiting translation of mRNA encoding a GABA<sub>B</sub>R2 polypeptide which comprises contacting such mRNA with an antisense oligonucleotide having a sequence capable of specifically hybridizing to the above-identified genomic DNA.

[0162] In one embodiment, the oligonucleotide comprises chemically modified nucleotides or nucleotide analogues.

[0163] In another embodiment, the isolated antibody is capable of binding to a GABA<sub>B</sub>R2 polypeptide encoded by an above-identified nucleic acid.

[0164] In another embodiment, the GABA<sub>B</sub>R2 polypeptide is a human GABA<sub>B</sub>R2 polypeptide.

[0165] This invention is directed to an antibody capable of competitively inhibiting the binding of an above-identified antibody to a GABA<sub>B</sub>R2 polypeptide.

[0166] In one embodiment, the antibody is a monoclonal antibody.

[0167] In one embodiment, the monoclonal antibody is directed to an epitope of a GABA<sub>B</sub>R2 polypeptide present on the surface of a GABA<sub>B</sub>R2 polypeptide expressing cell.

[0168] In another embodiment, the oligonucleotide is coupled to a substance which inactivates mRNA.

[0169] In another embodiment, the substance which inactivates mRNA is a ribozyme.

[0170] This invention is directed to a pharmaceutical composition which comprises an amount of an above-identified antibody effective to block binding of a ligand to the GABA<sub>B</sub>R2 polypeptide and a pharmaceutically acceptable carrier.

[0171] As used herein, "pharmaceutically acceptable carriers" means any of the standard pharmaceutically acceptable carriers. Examples include, but are not limited to, phosphate buffered saline, physiological saline, water and emulsions, such as oil/water emulsions.

[0172] Animal model systems which elucidate the physiological and behavioral roles of the polypeptides of this invention are produced by creating transgenic animals in which the activity of the polypeptide is either increased or decreased, or the amino acid sequence of the expressed polypeptide is altered, by a variety of techniques. Examples of these techniques include, but are not limited to: 1) Insertion of normal or mutant versions of DNA encoding the polypeptide, by microinjection, electroporation, retroviral transfection or other means well known to those skilled in the art, into appropriate fertilized embryos in order to produce a transgenic animal or 2) Homologous recombination of mutant or normal, human or animal versions of these genes with the native gene locus in transgenic animals to alter the regulation of expression or the structure of these polypeptide sequences. The technique of homologous recombination is well known in the art. It replaces the native gene with the inserted gene and so is useful for producing an animal that cannot express native polypeptides but does express, for example, an inserted mutant polypeptide, which has replaced the native polypeptide in the animal's genome by recombination, resulting in underexpression of the transporter. Microinjection adds genes to the genome, but does not remove them, and so is useful for producing an animal

which expresses its own and added polypeptides, resulting in overexpression of the polypeptides.

[0173] One means available for producing a transgenic animal, with a mouse as an example, is as follows: Female mice are mated, and the resulting fertilized eggs are dissected out of their oviducts. The eggs are stored in an appropriate medium such as M2 medium. DNA or cDNA encoding a polypeptide of this invention is purified from a vector by methods well known in the art. Inducible promoters may be fused with the coding region of the DNA to provide an experimental means to regulate expression of the trans-gene. Alternatively, or in addition, tissue specific regulatory elements may be fused with the coding region to permit tissue-specific expression of the trans-gene. The DNA, in an appropriately buffered solution, is put into a microinjection needle (which may be made from capillary tubing using a pipet puller) and the egg to be injected is put in a depression slide. The needle is inserted into the pronucleus of the egg, and the DNA solution is injected. The injected egg is then transferred into the oviduct of a pseudopregnant mouse (a mouse stimulated by the appropriate hormones to maintain pregnancy but which is not actually pregnant), where it proceeds to the uterus, implants, and develops to term. As noted above, microinjection is not the only method for inserting DNA into the egg cell, and is used here only for exemplary purposes.

[0174] This invention is directed to a transgenic, nonhuman mammal expressing DNA encoding a GABA<sub>n</sub>R2 polypeptide.

[0175] This invention is directed to a transgenic, nonhuman mammal comprising a homologous recombination knockout of the native GABA<sub>B</sub>R2 polypeptide.

[0176] This invention is further directed to a transgenic, nonhuman mammal whose genome comprises antisense DNA complementary to DNA encoding a GABA<sub>B</sub>R2 polypeptide so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding such GABA<sub>B</sub>R2 polypeptide and which hybridizes to such mRNA encoding such GABA<sub>B</sub>R2 polypeptide, thereby reducing its translation.

[0177] This invention is directed to an above-identified transgenic, nonhuman mammal, wherein the DNA encoding the GABA<sub>B</sub>R2 polypeptide additionally comprises an inducible promoter.

[0178] This invention is directed to an above-identified transgenic, nonhuman mammal, wherein the DNA encoding the GABA<sub>B</sub>R2 polypeptide additionally comprises tissue specific regulatory elements.

[0179] This invention is directed to an above-identified transgenic, nonhuman mammal, wherein the transgenic, nonhuman mammal is a mouse.

[0180] This invention is directed to method of detecting the presence of a GABA<sub>B</sub>R2 polypeptide on the surface of a cell which comprises contacting the cell with an above-identified antibody under conditions permitting binding of the antibody to the polypeptide, detecting the presence of the antibody bound to the cell, and thereby detecting the presence of a GABA<sub>B</sub>R2 polypeptide on the surface of the cell.

[0181] This invention is directed to a method of preparing a purified GABA<sub>n</sub>R2 polypeptide which comprises:

- [0182] a. inducing cells to express a GABA<sub>B</sub>R2 polypeptide;
- [0183] b. recovering the polypeptide so expressed from the induced cells; and
- [0184] c. purifying the polypeptide so recovered.

[0185] This invention is directed to a method of preparing the purified GABA<sub>B</sub>R2 polypeptide which comprises:

- [0186] a. inserting a nucleic acid encoding the GABA<sub>B</sub>R2 polypeptide into a suitable vector;
- [0187] b. introducing the resulting vector in a suitable host cell;
- [0188] c. placing the resulting cell in suitable condition permitting the production of the GABA<sub>B</sub>R2 polypeptide;
- [0189] d. recovering the polypeptide produced by the resulting cell; and
- [0190] e. isolating or purifying the polypeptide so recovered.

[0191] This invention is directed to a GABA<sub>B</sub>R1/R2 receptor comprising two polypeptides, one of which is a GABA<sub>B</sub>R2 polypeptide and another of which is a GABA<sub>B</sub>R1 polypeptide.

[0192] This invention is directed to a method of forming a GABA<sub>B</sub>R1/R2 receptor which comprises inducing cells to express both a GABA<sub>B</sub>R1 polypeptide and a GABA<sub>B</sub>R2 polypeptide.

[0193] GABA<sub>B</sub>R1 as used in this application could be GABA<sub>B</sub>R1a or GABA<sub>B</sub>R1b. The observation that at least two variants of the GABA<sub>B</sub>R1 polypeptide exist raises the possibility that GABA<sub>B</sub>R2 splice variants may exist or that there may exist introns in coding or non-coding regions of the genes encoding the GABA<sub>B</sub>R2 polypeptides. In addition, spliced form(s) of mRNA may encode additional amino acids either upstream of the currently defined starting methionine or within the coding region. Further, the existence and use of alternative exons is possible, whereby the mRNA may encode different amino acids within the region comprising the exon. In addition, single amino acid substitutions may arise via the mechanism of RNA editing such that the amino acid sequence of the expressed protein is different than that encoded by the original gene (Burns et al., 1996; Chu et al., 1996). Such variants may exhibit pharmacologic properties differing from the polypeptide encoded by the original gene.

[0194] The activity of a G-protein coupled receptor (GPCR) typically is measured using any of a variety of functional assays in which activation of the receptor in question results in an observable change in the level of some second messenger system, including but not limited to adenylate cyclase, calcium mobilization, arachidonic acid release, ion channel activity, inositol phospholipid hydrolysis or guanylyl cyclase. Heterologous expression systems utilizing appropriate host cells to express the nucleic acids of the subject invention are used to obtain the desired second messenger coupling. Receptor activity may also be assayed in an oocyte expression system.

[0195] The pharmacologic properties of the receptor described herein when GABA<sub>B</sub>R2 is co-expressed with

GABA<sub>B</sub>R1, are similar to the pharmacologic properties of the GABA<sub>B</sub> receptor observed using tissues. For convenience, in the context of the present invention applicants will refer to the product of the heterologous coexpression of GABA<sub>B</sub>R2 and GABA<sub>B</sub>R1 as the "GABA<sub>B</sub>R1/R2 receptor." Thus, a cell expressing nucleic acid encoding a GABA<sub>B</sub>R1/R2 receptor is to be understood to refer to a cell expressing both nucleic acid encoding a GABA<sub>B</sub>R1 polypeptide and nucleic acid encoding a GABA<sub>B</sub>R2 polypeptide. In this application, GABA<sub>B</sub>R1 can be either GABA<sub>B</sub>R1a or GABA<sub>B</sub>R1b.

[0196] This invention is directed to an antibody capable of binding to a GABA<sub>B</sub>R1/R2 receptor, wherein the GABA<sub>B</sub>R2 polypeptide is encoded by an above-identified nucleic acid.

[0197] This invention is directed to an above-identified antibody, wherein the GABA<sub>B</sub>R2 polypeptide is a human GABA<sub>B</sub>R2 polypeptide.

[0198] This invention is directed to an antibody capable of competitively inhibiting the binding of an above-identified antibody to a GABA<sub>B</sub>R1/R2 receptor.

[0199] In one embodiment, the antibody is a monoclonal antibody.

[0200] This invention is directed to an above-identified monoclonal antibody directed to an epitope of a GABA<sub>B</sub>R1/R2 receptor present on the surface of a GABA<sub>B</sub>R1/R2 polypeptide expressing cell.

[0201] This invention is directed to a pharmaceutical composition which comprises an amount of an above-identified antibody effective to block binding of a ligand to the GABA<sub>B</sub>R1/R2 receptor and a pharmaceutically acceptable carrier.

[0202] This invention is directed to a transgenic, nonhuman mammal expressing a GABA<sub>B</sub>R1/R2 receptor, which is not naturally expressed by the mammal.

[0203] This invention is directed to a transgenic, nonhuman mammal comprising a homologous recombination knockout of the native GABA<sub>B</sub>R1/R2 receptor.

[0204] In one embodiment, the transgenic nonhuman mammal is a mouse.

[0205] This invention is directed to a method of detecting the presence of a GABA<sub>B</sub>R1/R2 receptor on the surface of a cell which comprises contacting the cell with an above-identified antibody under conditions permitting binding of the antibody to the receptor, detecting the presence of the antibody bound to the cell, and thereby detecting the presence of a GABA<sub>B</sub>R1/R2 receptor on the surface of the cell.

[0206] This invention is directed to a method of determining the physiological effects of varying levels of activity of GABA<sub>B</sub>R1/R2 receptors which comprises producing an above-identified transgenic nonhuman mammal whose levels of GABA<sub>B</sub>R1/R2 receptor activity vary due to the presence of an inducible promoter which regulates GABA<sub>B</sub>R1/R2 receptor expression.

[0207] This invention is directed to a method of determining the physiological effects of varying levels of activity of GABA<sub>B</sub>R1/R2 receptors which comprises producing a panel

of above-identified transgenic nonhuman mammals, each expressing a different amount of GABA<sub>B</sub>R1/R2 receptor.

[0208] This invention is directed to a method for identifying an antagonist capable of alleviating an abnormality, by decreasing the activity of a GABA<sub>B</sub>R1/R2 receptor comprising administering a compound to a above-identified transgenic nonhuman mammal, and determining whether the compound alleviates the physical and behavioral abnormalities displayed by the transgenic, nonhuman mammal, the alleviation of the abnormality identifying the compound as the antagonist.

[0209] This invention is directed to an antagonist identified by an above-identified method.

[0210] This invention is directed to a pharmaceutical composition comprising an above-identified antagonist and a pharmaceutically acceptable carrier.

[0211] This invention is directed to a method of treating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of a GABA<sub>B</sub>R1/R2 receptor which comprises administering to a subject an effective amount of an above-identified pharmaceutical composition, thereby treating the abnormality.

[0212] This invention is directed to a method for identifying an agonist capable of alleviating an abnormality, by increasing the activity of a GABA<sub>B</sub>R1/R2 receptor comprising administering a compound to an above-identified transgenic nonhuman mammal, and determining whether the compound alleviates the physical and behavioral abnormalities displayed by the transgenic, nonhuman mammal, the alleviation of the abnormality identifying the compound as the agonist.

[0213] This invention is directed to an agonist identified by an above-identified method.

[0214] This invention is directed to a pharmaceutical composition comprising an above-identified agonist and a pharmaceutically acceptable carrier.

[0215] This invention is directed to a method for treating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a GABA<sub>B</sub>R1/R2 receptor which comprises administering to a subject an effective amount of an above-identified pharmaceutical composition, thereby treating the abnormality.

[0216] This invention is directed to a cell which expresses on its surface a mammalian GABA<sub>B</sub>R1/R2 receptor that is not naturally expressed on the surface of such cell.

[0217] This invention is directed to a cell, wherein the mammalian GABA<sub>B</sub>R1/R2 receptor comprises two polypeptides, one of which is a GABA<sub>B</sub>R2 polypeptide and another of which is a GABA<sub>B</sub>R1 polypeptide.

[0218] This invention is directed to a process for identifying a chemical compound which specifically binds to a GABA<sub>B</sub>R1/R2 receptor which comprises contacting cells containing nucleic acid encoding and expressing on their cell surface the GABA<sub>B</sub>R1/R2 receptor, wherein such cells do not normally express the GABA<sub>B</sub>R1/R2 receptor, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the GABA<sub>B</sub>R1/R2 receptor.

[0219] This invention is directed to a process for identifying a chemical compound which specifically binds to a GABA<sub>B</sub>R1/R2 receptor which comprises contacting a membrane fraction from a cell extract of cells containing nucleic acid encoding and expressing on their cell surface the GABA<sub>B</sub>R1/R2 receptor, wherein such cells do not normally express the GABA<sub>B</sub>R1/R2 receptor, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the GABA<sub>B</sub>R1/R2 receptor.

[0220] In one embodiment, the GABA<sub>B</sub>R1/R2 receptor is a mammalian GABA<sub>B</sub>R1/R2 receptor.

[0221] In another embodiment, the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has substantially the same amino acid sequence as that encoded by the plasmid BO-55 (ATCC Accession No. 209104).

[0222] In another embodiment, the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has substantially the same sequence as the amino acid sequence shown in FIGS. 23A-23D (SEQ ID NO:47).

[0223] In another embodiment, the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has the amino acid sequence shown in FIGS. 23A-23D (SEQ ID NO:47).

[0224] In another embodiment, the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has substantially the same amino acid sequence as that encoded by the plasmid pEXT3T7-hGABAB2 (ATCC Accession No. 203515).

[0225] In another embodiment, the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has substantially the same amino acid sequence as the sequence shown in FIGS. 23A-23D (SEQ ID NO:47).

[0226] In another embodiment, the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has the sequence shown in FIGS. 23A-23D (SEQ ID NO:47).

[0227] In another embodiment, the compound is not previously known to bind to a GABA<sub>B</sub>R1/R2 receptor.

[0228] This invention is directed to a compound identified by an above-identified process.

[0229] In one embodiment, the cell is an insect cell.

[0230] In another embodiment, the cell is a mammalian cell.

[0231] In another embodiment, the cell is nonneuronal in origin.

[0232] In another embodiment, the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell a mouse Y1 cell or LM(tk-) cell.

[0233] In another embodiment, the compound is not previously known to bind to a GABA<sub>B</sub>R1/R2 receptor.

[0234] This invention is directed to a compound identified by an above-identified process.

[0235] This invention is directed to a process involving competitive binding for identifying a chemical compound which specifically binds to a GABA<sub>B</sub>R1/R2 receptor which comprises separately contacting cells expressing on their cell surface the GABA<sub>B</sub>R1/R2 receptor, wherein such cells

do not normally express the GABA<sub>B</sub>R1/R2 receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the chemical compound to the GABA<sub>B</sub>R1/R2 receptor, a decrease in the binding of the second chemical compound to the GABA<sub>B</sub>R1/R2 receptor in the presence of the chemical compound indicating that the chemical compound binds to the GABA<sub>B</sub>R1/R2 receptor.

[0236] This invention is directed to a process involving competitive binding for identifying a chemical compound which specifically binds to a human GABA<sub>B</sub>R1/R2 receptor which comprises separately contacting a membrane fraction from a cell extract of cells expressing on their cell surface the GABA<sub>B</sub>R1/R2 receptor, wherein such cells do not normally express the GABA<sub>B</sub>R1/R2 receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the chemical compound to the GABA<sub>B</sub>R1/R2 receptor, a decrease in the binding of the second chemical compound to the GABA<sub>B</sub>R1/R2 receptor in the presence of the chemical compound indicating that the chemical compound binds to the GABA<sub>B</sub>R1/R2 receptor.

[0237] In one embodiment, the GABA<sub>B</sub>R1/R2 receptor is a mammalian GABA<sub>B</sub>R1/R2 receptor.

[0238] In another embodiment, the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has substantially the same amino acid sequence as that encoded by plasmid BO-55 (ATCC Accession No. 209104).

[0239] In another embodiment, the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has substantially the same amino acid sequence as that shown in FIGS. 23A-23D (SEQ ID NO:47).

[0240] In another embodiment, the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has the amino acid sequence shown in FIGS. 23A-23D (SEQ ID NO:47).

[0241] In another embodiment, the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has substantially the same amino acid sequence as that encoded by plasmid pEXT3T7-hGABAB2 (ATCC Accession No. 203515).

[0242] In another embodiment, the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has substantially the same amino acid sequence as the sequence shown in FIGS. 23A-23D (SEQ ID NO:47).

[0243] In another embodiment, the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has the sequence shown in FIGS. 23A-23D (SEQ ID NO:47).

[0244] In another embodiment, the cell is an insect cell.

[0245] In another embodiment, the cell is a mammalian cell.

[0246] In another embodiment, the cell is nonneuronal in origin.

[0247] In another embodiment, the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell a mouse Y1 cell or LM(tk-) cell.

[0248] In another embodiment, the compound is not previously known to bind to a GABA<sub>B</sub>R1/R2 receptor.

[0249] This invention is directed to a compound identified by an above-identified process.

[0250] This invention is directed to a method of screening a plurality of chemical compounds not known to bind to a GABA<sub>B</sub>R1/R2 receptor to identify a compound which specifically binds to the GABA<sub>B</sub>R1/R2 receptor, which comprises

[0251] (a) contacting cells containing nucleic acid encoding and expressing on their cell surface the GABA<sub>B</sub>R1/R2 receptor, wherein such cells do not normally express the GABA<sub>B</sub>R1/R2 receptor, with a compound known to bind specifically to the GABA<sub>B</sub>R1/R2 receptor;

[0252] (b) contacting the same cells as in step (a) with the plurality of compounds not known to bind specifically to the GABA<sub>B</sub>R1/R2 receptor, under conditions permitting binding of compounds known to bind the GABA<sub>B</sub>R1/R2 receptor;

[0253] (c) determining whether the binding of the compound known to bind specifically to the GABA<sub>B</sub>R1/R2 receptor is reduced in the presence of the plurality of the compounds, relative to the binding of the compound in the absence of the plurality of compounds, and if the binding is reduced;

[0254] (d) separately determining the extent of binding to the GABA<sub>B</sub>R1/R2 receptor of each compound included in the plurality of compounds, so as to thereby identify the compound or compounds present in such plurality of compounds which specifically binds to the GABA<sub>B</sub>R1/R2 receptor.

[0255] This invention is directed to a method of screening a plurality of chemical compounds not known to bind to a GABA<sub>B</sub>R1/R2 receptor to identify a compound which specifically binds to the GABA<sub>B</sub>R1/R2 receptor, which comprises

[0256] (a) contacting a membrane fraction extract from cells containing nucleic acid encoding and expressing on their cell surface the GABA<sub>B</sub>R1/R2 receptor, wherein such cells do not normally express the GABA<sub>B</sub>R1/R2 receptor, with a compound known to bind specifically to the GABA<sub>B</sub>R1/R2 receptor;

[0257] (b) contacting the same membrane fraction as in step (a) with the plurality of compounds not known to bind specifically to the GABA<sub>B</sub>R1/R2 receptor, under conditions permitting binding of compounds known to bind the GABA<sub>B</sub>R1/R2 receptor;

[0258] (c) determining whether the binding of the compound known to bind specifically to the GABA<sub>B</sub>R1/R2 receptor is reduced in the presence of the plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds, and if the binding is reduced;

[0259] (d) separately determining the extent of binding to the GABA<sub>B</sub>R1/R2 receptor of each compound included in the plurality of compounds, so as to

thereby identify the compound or compounds present in such plurality of compounds which specifically binds to the GABA<sub>B</sub>R1/R2 receptor.

[0260] In one embodiment, the GABA<sub>B</sub>R1/R2 receptor is a mammalian GABA<sub>B</sub>R1/R2 receptor.

[0261] In one embodiment, the cell is a mammalian cell.

[0262] In one embodiment, the mammalian cell is non-neuronal in origin.

[0263] In one embodiment, the non-neuronal cell is a COS-7 cell, a 293 human embryonic kidney cell, a LM(tk-) cell, a CHO cell, a mouse Y1 cell or an NIH-3T3 cell.

[0264] This invention is directed to a process for determining whether a chemical compound is a GABA<sub>B</sub>R1/R2 receptor agonist which comprises contacting cells with the compound under conditions permitting the activation of the GABA<sub>B</sub>R1/R2 receptor, and detecting an increase in GABA<sub>B</sub>R1/R2 receptor activity, so as to thereby determine whether the compound is a GABA<sub>B</sub>R1/R2 receptor agonist.

[0265] This invention is directed to a process for determining whether a chemical compound is a GABA<sub>B</sub>R1/R2 receptor antagonist which comprises contacting cells containing nucleic acid encoding and expressing on their cell surface the GABA<sub>B</sub>R1/R2 receptor, wherein such cells do not normally express the GABA<sub>B</sub>R1/R2 receptor, with the compound in the presence of a known GABA<sub>B</sub>R1/R2 receptor agonist, under conditions permitting the activation of the GABA<sub>B</sub>R1/R2 receptor, and detecting a decrease in GABA<sub>B</sub>R1/R2 receptor activity, so as to thereby determine whether the compound is a GABA<sub>B</sub>R1/R2 receptor antagonist.

[0266] Expression of genes in *Xenopus* oocytes is well known in the art (A. Coleman, *Transcription and Translation: A Practical Approach* (B. D. Hanes, S. J. Higgins, eds., pp 271-302, IRL Press, Oxford, 1984; Y. Masu et al., *Nature* 329:21583-21586, 1994) and is performed using microinjection of native mRNA or in vitro synthesized mRNA into frog oocytes. The preparation of in vitro synthesized mRNA can be performed by various standard techniques (J. Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1989) including using T7 polymerase with the mCAP RNA capping kit (Stratagene).

[0267] In one embodiment, the cells additionally express nucleic acid encoding GIRK1 and GIRK4.

[0268] In another embodiment, the GABA<sub>B</sub>R2 receptor is a mammalian GABA<sub>B</sub>R2 receptor.

[0269] This invention is directed to a pharmaceutical composition which comprises an amount of a GABA<sub>B</sub>R1/R2 receptor agonist determined to be an agonist by an above-identified process effective to increase activity of a GABA<sub>B</sub>R1/R2 receptor and a pharmaceutically acceptable carrier.

[0270] This invention is directed to a pharmaceutical, wherein the GABA<sub>B</sub>R1/R2 receptor agonist was not previously known.

[0271] This invention is directed to a pharmaceutical composition which comprises an amount of a GABA<sub>B</sub>R1/R2 receptor antagonist determined to be an antagonist an

above-identified process effective to reduce activity of a GABA<sub>B</sub>R1/R2 receptor and a pharmaceutically acceptable carrier.

[0272] This invention is directed to a pharmaceutical composition, wherein the GABA<sub>B</sub>R1/R2 receptor antagonist was not previously known.

[0273] This invention is directed to a process for determining whether a chemical compound activates a GABA<sub>B</sub>R1/R2 receptor, which comprises contacting cells producing a second messenger response and expressing on their cell surface the GABA<sub>B</sub>R1/R2 receptor, wherein such cells do not normally express the GABA<sub>B</sub>R1/R2 receptor, with the chemical compound under conditions suitable for activation of the GABA<sub>B</sub>R1/R2 receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change in the second messenger response in the presence of the chemical compound indicating that the compound activates the GABA<sub>B</sub>R1/R2 receptor.

[0274] In one embodiment, the second messenger response comprises potassium channel activation and the change in second messenger is an increase in the level of potassium current.

[0275] This invention is directed to a process for determining whether a chemical compound inhibits activation of a GABA<sub>B</sub>R1/R2 receptor, which comprises separately contacting cells producing a second messenger response and expressing on their cell surface the GABA<sub>B</sub>R1/R2 receptor, wherein such cells do not normally express the GABA<sub>B</sub>R1/R2 receptor, with both the chemical compound and a second chemical compound known to activate the GABA<sub>B</sub>R1/R2 receptor, and with only the second chemical compound, under conditions suitable for activation of the GABA<sub>B</sub>R1/R2 receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change in the second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the GABA<sub>B</sub>R1/R2 receptor.

[0276] In one embodiment, the second messenger response comprises potassium channel activation and the change in second messenger response is a smaller increase in the level of inward potassium current in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

[0277] This invention is directed to an above-identified process, wherein the GABA<sub>B</sub>R1/R2 receptor is a mammalian GABA<sub>B</sub>R1/R2 receptor.

[0278] In one embodiment, the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has substantially the same amino acid sequence as that encoded by the plasmid BO-55 (ATCC Accession No. 209104).

[0279] In another embodiment, the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has substantially the same amino acid sequence as that shown in FIGS. 4A-4D (SEQ ID NO:4).

[0280] In another embodiment, the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has substantially the same amino acid sequence as that shown in FIGS. 23A-23D (SEQ ID NO:47).

[0281] In another embodiment, the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has the sequence, shown in FIGS. 23A-23D (SEQ ID NO:47).

[0282] In another embodiment, the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has substantially the same amino acid sequence as that encoded by the plasmid pEXJT3T7-hGABAB2 (ATCC Accession No. 203515).

[0283] This invention is directed to an above-identified process, wherein the cell is an insect cell.

[0284] This invention is directed to an above-identified process, wherein the cell is a mammalian cell.

[0285] In one embodiment, the mammalian cell is non-neuronal in origin.

[0286] In another embodiment, the nonneuronal cell is a COS-7 cell, CHO cell, 293 human embryonic kidney cell, NIH-3T3 cell or LM(tk-) cell.

[0287] In another embodiment, the compound was not previously known to activate or inhibit a GABA<sub>B</sub>R1/R2 receptor.

[0288] This invention is directed to a compound determined by an above-identified process.

[0289] This invention is directed to a pharmaceutical composition which comprises an amount of a GABA<sub>B</sub>R1/R2 receptor agonist determined by an above-identified process effective to increase activity of a GABA<sub>B</sub>R1/R2 receptor and a pharmaceutically acceptable carrier.

[0290] In one embodiment, the GABA<sub>B</sub>R1/R2 receptor agonist was not previously known.

[0291] This invention is directed to a pharmaceutical composition which comprises an amount of a GABA<sub>B</sub>R1/R2 receptor antagonist determined by an above-identified process effective to reduce activity of a GABA<sub>B</sub>R1/R2 receptor and a pharmaceutically acceptable carrier.

[0292] In one embodiment, the GABA<sub>B</sub>R1/R2 receptor antagonist was not previously known.

[0293] This invention is directed to method of screening a plurality of chemical compounds not known to activate a GABA<sub>B</sub>R1/R2 receptor to identify a compound which activates the GABA<sub>B</sub>R1/R2 receptor which comprises:

[0294] (a) contacting cells containing nucleic acid encoding and expressing on their cell surface the GABA<sub>B</sub>R1/R2 receptor, wherein such cells do not normally express the GABA<sub>B</sub>R1/R2 receptor, with the plurality of compounds not known to activate the GABA<sub>B</sub>R1/R2 receptor, under conditions permitting activation of the GABA<sub>B</sub>R1/R2 receptor;

[0295] (b) determining whether the activity of the GABA<sub>B</sub>R1/R2 receptor is increased in the presence of the compounds, and if it is increased;

[0296] (c) separately determining whether the activation of the GABA<sub>B</sub>R1/R2 receptor is increased by

each compound included in the plurality of compounds, so as to thereby identify the compound or compounds present in such plurality of compounds which activates the GABA<sub>B</sub>R1/R2 receptor.

[0297] In one embodiment, the cells express nucleic acid encoding GIRK1 and GIRK4.

[0298] In another embodiment, the GABA<sub>B</sub>R1/R2 receptor is a mammalian GABA<sub>B</sub>R1/R2 receptor.

[0299] This invention is directed to a method of screening a plurality of chemical compounds not known to inhibit the activation of a GABA<sub>B</sub>R1/R2 receptor to identify a compound which inhibits the activation of the GABA<sub>B</sub>R1/R2 receptor, which comprises:

[0300] (a) contacting cells containing nucleic acid encoding and expressing on their cell surface the GABA<sub>B</sub>R1/R2 receptor, wherein such cells do not normally express the GABA<sub>B</sub>R1/R2 receptor, with the plurality of compounds in the presence of a known GABA<sub>B</sub>R1/R2 receptor agonist, under conditions permitting activation of the GABA<sub>B</sub>R1/R2 receptor;

[0301] (b) determining whether the activation of the GABA<sub>B</sub>R1/R2 receptor is reduced in the presence of the plurality of compounds, relative to the activation of the GABA<sub>B</sub>R1/R2 receptor in the absence of the plurality of compounds, and if it is reduced;

[0302] (c) separately determining the inhibition of activation of the GABA<sub>B</sub>R1/R2 receptor for each compound included in the plurality of compounds, so as to thereby identify the compound or compounds present in such a plurality of compounds which inhibits the activation of the GABA<sub>B</sub>R1/R2 receptor.

[0303] In one embodiment, the cells express nucleic acid encoding GIRK1 and GIRK4.

[0304] In one embodiment, the GABA<sub>B</sub>R1/R2 receptor is a mammalian GABA<sub>B</sub>R1/R2 receptor.

[0305] In another embodiment, wherein the cell is a mammalian cell.

[0306] In another embodiment, the mammalian cell is non-neuronal in origin.

[0307] In another embodiment, the non-neuronal cell is a COS-7 cell, a 293 human embryonic kidney cell, a LM(tk-) cell or an NIH-3T3 cell.

[0308] This invention is directed to a pharmaceutical composition comprising a compound identified by an above-identified method, effective to increase GABA<sub>B</sub>R1/R2 receptor activity and a pharmaceutically acceptable carrier.

[0309] This invention is directed to a pharmaceutical composition comprising a compound identified by an above-identified method, effective to decrease GABA<sub>B</sub>R1/R2 receptor activity and a pharmaceutically acceptable carrier.

[0310] This invention is directed to a process for determining whether a chemical compound is a GABA<sub>B</sub>R1/R2 receptor agonist, which comprises preparing a membrane fraction from cells which comprise nucleic acid encoding and expressing on their cell surface the GABA<sub>B</sub>R1/R2

receptor, wherein such cells do not normally express the GABA<sub>B</sub>R1/R2 receptor, separately contacting the membrane fraction with both the chemical compound and GTPYS, and with only GTPYS, under conditions permitting the activation of the GABA<sub>B</sub>R1/R2 receptor, and detecting GTPYS binding to the membrane fraction, an increase in GTPYS binding in the presence of the compound indicating that the chemical compound activates the GABA<sub>B</sub>R1/R2 receptor.

[0311] This invention is directed to a process for determining whether a chemical compound is a GABA<sub>B</sub>R1/R2 receptor antagonist, which comprises preparing a membrane fraction from cells which comprise nucleic acid encoding and expressing on their cell surface the GABA<sub>B</sub>R1/R2 receptor, wherein such cells do not normally express the GABA<sub>B</sub>R1/R2 receptor, separately contacting the membrane fraction with the chemical compound, GTPYS and a second chemical compound known to activate the GABA<sub>B</sub>R1/R2 receptor, with GTPYS and only the second compound, and with GTPYS alone, under conditions permitting the activation of the GABA<sub>B</sub>R1/R2 receptor, detecting GTPYS binding to each membrane fraction, and comparing the increase in GTPYS binding in the presence of the compound and the second compound relative to the binding of GTPYS alone, to the increase in GTPYS binding in the presence of the second chemical compound known to activate the GABA<sub>B</sub>R1/R2 receptor relative to the binding of GTPYS alone, a smaller increase in GTPYS binding in the presence of the compound and the second compound indicating that the compound is a GABA<sub>B</sub>R1/R2 receptor antagonist.

[0312] In one embodiment, the GABA<sub>B</sub>R2 receptor is a mammalian GABA<sub>B</sub>R2 receptor.

[0313] In another embodiment, the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has substantially the same amino acid sequence as that encoded by the plasmid BO-55 (ATCC Accession No. 209104).

[0314] In another embodiment, the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has substantially the same amino acid sequence as that shown in FIGS. 4A-4D (SEQ ID NO:4).

[0315] In another embodiment, the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has substantially the same amino acid sequence as that encoded by the plasmid pEXT3T7-hGABAB2 (ATCC Accession No. 203515).

[0316] In another embodiment, the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has substantially the same amino acid sequence as that shown in FIGS. 23A-23D (SEQ ID NO:47).

[0317] In another embodiment, the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has the sequence shown in FIGS. 23A-23D (SEQ ID NO:47).

[0318] In another embodiment, the cell is an insect cell.

[0319] In another embodiment, the cell is a mammalian cell.



[0320] In another embodiment, the mammalian cell is nonneuronal in origin.

[0321] In another embodiment, the nonneuronal cell is a COS-7 cell, CHO cell, 293 human embryonic kidney cell, NIH-3T3 cell or LM(tk-) cell.

[0322] In another embodiment, the compound was not previously known to be an agonist or antagonist of a GABA<sub>B</sub>R1/R2 receptor.

[0330] This invention directed to a method of treating Alzheimer's disease in a subject which comprises administering to the subject an amount of a compound which is a GABA<sub>B</sub>R1/R2 receptor antagonist effective to treat Alzheimer's disease in the subject.

[0331] This invention is directed to a peptide selected from the group consisting of:

- a) P L Y S I L S A L T I L G M I M A S A F L F F N I (SEQ ID NO:48)  
K N;
- b) L I I L G G M L S Y A S I F L F G L D G S F V S E (SEQ ID NO:49)  
K T;
- c) C T V R T W I L T V G Y T T A F G A M F A K T W (SEQ ID NO:50)  
R;
- d) Q K L L V I V G G M L L I D L C L L I C W Q; (SEQ ID NO:51)
- e) M T I W L G I V Y A Y K G L L M L F G C F L A (SEQ ID NO:52)  
W;
- f) A L N D S K Y I G M S V Y N V G I M C I I G A A V; (SEQ ID NO:53) and
- g) C I V A L V I I F C S T I T L C L V F V P K L I T (SEQ ID NO:54)  
L R T N.

[0323] This invention is directed to a compound determined to be an agonist or antagonist of a GABA<sub>B</sub>R1/R2 receptor by an above-identified process.

[0324] This invention is directed to a method of treating spasticity in a subject which comprises administering to the subject an amount of a compound which is an agonist of a GABA<sub>B</sub>R1/R2 receptor effective to treat spasticity in the subject.

[0325] This invention is directed to a method of treating asthma in a subject which comprises administering to the subject an amount of a compound which is a GABA<sub>B</sub>R1/R2 receptor agonist effective to treat asthma in the subject.

[0326] This invention is directed to a method of treating incontinence in a subject which comprises administering to the subject an amount of a compound which is a GABA<sub>B</sub>R1/R2 receptor agonist effective to treat incontinence in the subject.

[0327] This invention is directed to method of decreasing nociception in a subject which comprises administering to the subject an amount of a compound which is a GABA<sub>B</sub>R1/R2 receptor agonist effective to decrease nociception in the subject.

[0328] This invention is directed to a use of a GABA<sub>B</sub>R2 agonist as an antitussive agent which comprises administering to the subject an amount of a compound which is a GABA<sub>B</sub>R1/R2 receptor agonist effective as an antitussive agent in the subject.

[0329] This invention is directed to a method of treating drug addiction in a subject which comprises administering to the subject an amount of a compound which is a GABA<sub>B</sub>R1/R2 receptor agonist effective to treat drug addiction in the subject.

[0332] This invention is directed to a compound that prevents the formation of a GABA<sub>B</sub>R1/R2 receptor complex.

[0333] Transmembrane peptides derived from GABA<sub>B</sub>R2 sequences may modulate the functional activity of GABA<sub>B</sub>R1/R2 receptors. One mode of action involves the destruction of the GABA<sub>B</sub>R1/R2 receptor complex via competitive displacement of the GABA<sub>B</sub>R2 polypeptide subunit by the peptide upon binding to the GABA<sub>B</sub>R1 polypeptide subunit. The peptides may be synthesized using standard solid phase F-moc peptide synthesis protocol using an Advanced Chemtech 396 Automated Peptide Synthesizer.

[0334] Additional GABA<sub>B</sub> subtypes in hypothalamus and caudate putamen are predicted due to the under-representation of GABA<sub>B</sub>R2 hybridization signals. These novel GABA<sub>B</sub> proteins and others may be identified by using GABA<sub>B</sub>R2 polypeptides in co-immunoprecipitation experiments.

[0335] This invention provides a process for making a composition of matter which specifically binds to a GABA<sub>B</sub>R1/R2 receptor which comprises identifying a chemical compound using any of the processes described herein for identifying a compound which binds to and/or activates or inhibits activation of a GABA<sub>B</sub>R1/R2 receptor and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof. In one embodiment, the GABA<sub>B</sub>R1/R2 receptor is a human GABA<sub>B</sub>R1/R2 receptor.

[0336] This invention further provides a process for preparing a pharmaceutical composition which comprises admixing a pharmaceutically acceptable carrier and a pharmaceutically acceptable amount of a chemical compound identified by any of the processes described herein for identifying a compound which binds to and/or activates or inhibits activation of a GABA<sub>B</sub>R1/R2 receptor or a novel

structural and functional analog or homolog thereof. In one embodiment, the GABA<sub>B</sub>R1/R2 receptor is a human GABA<sub>B</sub>R1/R2 receptor.

[0337] Thus, once the gene for a targeted receptor subtype is cloned, it is placed into a recipient cell which then expresses the targeted receptor subtype on its surface. This cell, which expresses a single population of the targeted human receptor subtype, is then propagated resulting in the establishment of a cell line. This cell line, which constitutes a drug discovery system, is used in two different types of assays: binding assays and functional assays. In binding assays, the affinity of a compound for both the receptor subtype that is the target of a particular drug discovery program and other receptor subtypes that could be associated with side effects are measured. These measurements enable one to predict the potency of a compound, as well as the degree of selectivity that the compound has for the targeted receptor subtype over other receptor subtypes. The data obtained from binding assays also enable chemists to design compounds toward or away from one or more of the relevant subtypes, as appropriate, for optimal therapeutic efficacy. In functional assays, the nature of the response of the receptor subtype to the compound is determined. Data from the functional assays show whether the compound is acting to inhibit or enhance the activity of the receptor subtype, thus enabling pharmacologists to evaluate compounds rapidly at their ultimate human receptor subtypes targets permitting chemists to rationally design drugs that will be more effective and have fewer or substantially less severe side effects than existing drugs.

[0338] Approaches to designing and synthesizing receptor subtype-selective compounds are well known and include traditional medicinal chemistry and the newer technology of combinatorial chemistry, both of which are supported by computer-assisted molecular modeling. With such approaches, chemists and pharmacologists use their knowledge of the structures of the targeted receptor subtype and compounds determined to bind and/or activate or inhibit activation of the receptor subtype to design and synthesize structures that will have activity at these receptor subtypes.

[0339] Combinatorial chemistry involves automated synthesis of a variety of novel compounds by assembling them using different combinations of chemical building blocks. The use of combinatorial chemistry greatly accelerates the

analogs are prepared to facilitate an understanding of the relationship between chemical structure and biological or functional activity. These studies define structure activity relationships which are then used to design drugs with improved potency, selectivity and pharmacokinetic properties. Combinatorial chemistry is also used to rapidly generate a variety of structures for lead optimization. Traditional medicinal chemistry, which involves the synthesis of compounds one at a time, is also used for further refinement and to generate compounds not accessible by automated techniques. Once such drugs are defined the production is scaled up using standard chemical manufacturing methodologies utilized throughout the pharmaceutical and chemistry industry.

[0341] This invention will be better understood from the Experimental Details which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims which follow thereafter.

#### Experimental Details

##### Materials and Methods

##### DNA Sequencing

[0342] DNA sequences were determined using an ABI PRISM 377 DNA Sequencer (Perkin-Elmer, Foster City, Calif.) according to the manufacturer's instructions.

##### Hybridization Methodology

[0343] Probes were end-labeled with polynucleotide kinase according to the manufacturer's instructions (Boehringer-Mannheim). Hybridization was performed on Zeta-Probe membrane (Bio-Rad, CA) at reduced stringency: 40° C. in a solution containing 25% formamide, 5×SSC (1×SSC=0.15 M NaCl, 0.015 M sodium citrate), 1×Denhardt's solution (0.02% polyvinylpyrrolidone, 0.02% Ficoll, 0.02% bovine serum albumin) and 25 µg/µL sonicated salmon sperm DNA. Membrane strips were washed at 40° C. in 0.1×SSC containing 0.1% SDS and exposed at -70° C. to Kodak XAR film in the presence of an intensifying screen.

[0344] The nucleotide sequences of the hybridization probes are shown below:

T-891: 5'-AGGGATGCTTCTCTATGCTTCCATATTCTCTTTGGCCCTGATGG-3' (SEQ ID NO:5)  
Nucleotides 1449-1493 of TL-267, forward strand.

T-892: 5'-CAATGTGCAGTCTGTCATCGTGGCTCTGGTCATCATCTTCTGCAG-3' (SEQ ID NO:6)  
Nucleotides 2022-2066 of TL-267, forward strand.

process of generating compounds. The resulting arrays of compounds are called libraries and are used to screen for compounds (lead compounds) that demonstrate a sufficient level of activity at receptors of interest. Using combinatorial chemistry it is possible to synthesize focused libraries of compounds anticipated to be highly biased toward the receptor target of interest.

[0340] Once lead compounds are identified, whether through the use of combinatorial chemistry or traditional medicinal chemistry or otherwise, a variety of homologs and

##### PCR Methodology

[0345] PCR reactions were carried out using a PE 9600 (Perkin-Elmer) PCR cycler in 20 µL volumes using Expand Long Template Polymerase (Boehringer-Mannheim) and the manufacturer's buffer 1 for internal PCR primers or manufacturer's buffer 2 for vector-anchored PCR. Reactions were run using a program consisting of 35 cycles of 94° C. for 30 sec., 68° C. for 20 sec, and 72° C. for 1 min, with a pre-incubation at 95° C. for 5 min and post-incubation hold at 4° C.

[0346] Nucleotide sequences of the primer sets used in PCR reactions are shown below:

T-94: 5'-CTTCTAGGCCTGTACGGAAGTGT-3' (SEQ ID NO: 7);  
vector, forward primer.

T-95: 5'-GTTGTGGTTTGTCCAACTCATCAAT-3' (SEQ ID NO: 8);  
vector, reverse primer.

T-887: 5'-GGGATGAGTGTCTACAACGTGGG-3' (SEQ ID NO: 9);  
nucleotides 1948-1971 of TL-267, forward9);  
primer.

T-888: 5'-TGCCTGCTGCATCTGGGTTTGTCT-3' (SEQ ID NO: 10);  
nucleotides 2138-2113 of TL-267, reverse10);  
primer.

T-889: 5'-ATCTCCCTACCTCTCTACAGCATCCT-3' (SEQ ID NO: 11);  
nucleotides 1300-1325 of TL-267, forward11);  
primer.

T-890: 5'-CAGGTCTGACGGTGCAAAGTGTTC-3' (SEQ ID NO: 12);  
nucleotides 1544-1519 of TL-267, reverse12);  
primer.

T-921: 5'-TGACGCAAGACGTTGAGGTTCTCT-3' (SEQ ID NO: 13);  
nucleotides 473-498 of TL-267, forward 13);  
primer.

T-922: 5'-TGTCAGCTTCCATGGCAGCAAGCAGA-3' (SEQ ID NO: 14);  
nucleotides 814-789 of TL-267, reverse 14);  
primer.

T-923: 5'-AGAGAACCTCTGAACGCTCTTGGCTCA-3' (SEQ ID NO: 15);  
nucleotides 498-473 of TL-267, reverse 15);  
primer.

T-935: 5'-GGCTCTGTTGTGTTCCACTGTAGCTG-3' (SEQ ID NO: 16);  
nucleotides 2483-2458 of TL-267, reverse16);  
primer.

T-938: 5'-TCATGC-  
CGCTCAC-  
CAAGGAGGTG-  
GCC-3' (SEQ ID NO: 17);  
nucleotides 53 to 78 of TL-267, forward 17);  
primer.

T-939: 5'-GGCCACCTCCTTGGTGAGCGGCATGA-3' (SEQ ID NO: 18);  
nucleotides 78 to 53 of TL-267, reverse 18);  
primer.

T-947: 5'-TGAGTGAGCAGAGTCCAGAGCCGT-3' (SEQ ID NO: 19);  
nucleotides -68 to -45 of TL-267, forward 19);  
ward primer.

T-948: 5'-ATGGATGGGAGGTAGGCGTGGTGAG-3' (SEQ ID NO: 20);  
nucleotides 2591-2566 of TL-267, reverse20);  
primer.

#### Preparation of Human Hippocampal cDNA Library

[0347] Total RNA was prepared by a modification of the guanidine thiocyanate method, from 6 grams of human hippocampus. Poly A<sup>+</sup>RNA was purified with a FastTrack kit (Invitrogen Corp., San Diego, Calif.). Double stranded (ds) cDNA was synthesized from 4 µg of poly A<sup>+</sup> RNA according to Gübler and Hoffman (1983), except that ligase was omitted in the second strand cDNA synthesis. The resulting DS cDNA was ligated to BstXI/EcoRI adaptors (Invitrogen Corp.), the excess of adaptors was removed by exclusion chromatography. High molecular weight fractions were ligated in pcEXV.BS (An Okayama and Berg expression vector) cut by BstXI as described by Aruffo and Seed (1987). The ligated DNA was electroporated in *E. coli* MC 1061 (Gene Pulser, Biorad). A total of 2.2×10<sup>6</sup> independent

clones with an insert mean size of approximately 3 kb was generated. The library was plated on Petri dishes (Ampicillin selection) in pools of 0.4 to 1.2×10<sup>4</sup> independent clones. After 18 hours amplification, the bacteria from each pool were scraped, resuspended in 4 mL of LB media and 1.5 mL processed for plasmid purification by the alkali method (Sambrook et al, 1989). 1 mL aliquots of each bacterial pool were stored at -85° C. in 20% glycerol.

#### BLAST Search that Identified a Novel 7-TM Protein Sequence

[0348] Sequence analysis was performed with the Wisconsin Package Version 9.0, Genetics Computer Group (GCG), Madison, Wis. The rat GABA<sub>B</sub>R1a amino acid sequence (Kaupmann et al. (1997) Nature 386:239) was used as a query to search the EST division of GenBank with BLAST. Two entries, T07621 and Z43654, had probability scores that suggested significant amino acid homology to the GABA<sub>B</sub>R1a polypeptide. T07621 had sequence homology from the beginning of the first transmembrane domain to the beginning of third transmembrane domain of the GABA<sub>B</sub>R1a polypeptide. Z43654 had sequence homology from the sixth transmembrane domain to the seventh transmembrane domain of the GABA<sub>B</sub>R1a polypeptide. The sequence documentation for T07621 and Z43654 was retrieved with Entrez (NCBI) and neither sequence was annotated as having homology to any 7-TM spanning protein.

#### T07621 and Z43654 are Part of the Same Sequence

[0349] A series of PCR reactions were carried out on human hippocampus DNA with multiple primer sets: primer set T-887/T-888 designed to Z43654 sequence; primer set T-889/T-890 designed to the T07621 sequence; and primer set T-889/T-888 designed to the forward strand of T07621 and the reverse stand of Z43654. The PCR products was loaded on duplicate lanes of an agarose gel and the DNA was southern blotted to a Zeta-Probe membrane (Bio-Rad, CA). The regions of the membrane corresponding to the individual lanes on the gel were cut to produce membrane strips that contained duplicate samples of the DNA. One set of membrane strips was hybridized with T-891, a probe specific for the T07621 sequence. Another set of membranes was hybridized with T-892, a probe specific to the Z43654 sequence. The membrane from primer set T-887/T-888 hybridized with probe T-892 for the Z43654 sequence. The membrane from primer set T-889/T-890 hybridized with probe T-891 for the T07621 sequence. The membrane from primer set T-889/T-888 hybridized with both the T-891 and T-892 probes.

#### Isolating the Full-length Human cDNA by PCR Sib Selection

[0350] PCR reactions were carried out on bacterial pools containing a human hippocampus cDNA library. Primer set T-888/T-889 was used to identify the bacterial pools that contained a portion of the novel receptor. Vector-anchored PCR was carried out on the positive pools to determine which pool contained the longest cDNA insert. Four primer sets were used for the vector-anchored PCR: T-94/T-888, T-94/T-889, T-95/T-888, and T-95/T-889. Pool 365 was identified having the longest cDNA insert and the plasmid was sib selected (McCormick, 1987). The nucleotide sequence of

clone 365-9-7-4, designated TL-260, was translated into amino acids and compared to the amino acid sequence of the rat GABA<sub>B</sub>R1a polypeptide. Relative to the rat GABA<sub>B</sub>R1a amino acid sequence, TL-260 was truncated at the amino terminus.

[0351] A set of PCR primers (T-921/T-922) was made to the 5' region of TL-260 and was used to re-screen the bacterial pools of the human hippocampus library for the missing segment of the novel clone. Vector-anchored PCR was carried out on the positive pools to determine which pool contained the longest cDNA insert. Four primer sets were used for the vector-anchored PCR: T-94/T-921, T-94/T-922, T-95/T-921, and T-95/T-922. Pool 299 contained the most 5' sequence. A PCR product derived from the primer set T-94/T-923 was isolated (T-261) and sequenced. The putative amino acids derived from TL-261 were compared to the rat GABA<sub>B</sub>R1 sequence. TL-261 contained an initiation codon but didn't contain a stop codon upstream of the initiation codon.

[0352] A set of PCR primers (T-938/T-935) was made to the 5' region of TL-261 and was used to re-screen the bacterial pools of the human hippocampus library for additional sequence. Vector-anchored PCR was carried out on the positive pools to determine which pool contained the longest cDNA insert. Four primer sets were used for the vector-anchored PCR: T-94/T-938, T-94/T-939, T-95/T-938, and T-95/T-939. A PCR product derived from primer set T-95/T-939 was isolated (T-261a) and sequenced. The putative amino acids derived from T-261a were compared to the rat GABA<sub>B</sub>R1 amino acid sequence. T-261a contained an initiation codon and an in-frame upstream stop codon.

[0353] From the vector-anchored PCR, pool 389 contained the longest cDNA insert. This pool was sub-selected with the primer set T-947/T-935. The resulting plasmid, 389-20-29-2, was designated TL-266 and was sequenced.

#### Construction of GABA<sub>B</sub>R2 Polypeptide in Expression Vector

[0354] A Cla-I-Xba-I fragment from TL-266 was sub-cloned into the expression vector pEXJ.HRT3T7 and designated TL-267. This plasmid (TL-267) was deposited on Jun. 10, 1997, with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Md. 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and was accorded ATCC Accession No. 209103.

#### Generation of Rat GABA<sub>B</sub>R2 PCR Product

[0355] cDNA from rat hippocampus and rat cerebellum were amplified in 50  $\mu$ L PCR reaction mixtures using the Expand Long Template PCR System (as supplied and described by the manufacturer, Boehringer Mannheim) using a program consisting of 40 cycles of 94° C. for 1 min, 50° C. for 2 min, and 68° C. for 2 min, with a pre- and post-incubation of 95° C. for 5 min and 68° C. for 7 min, respectively. PCR primers for rat GABA<sub>B</sub>R2 were designed against the human GABA<sub>B</sub>R2 sequence: BB 257, forward primer in the first transmembrane domain, and BB 258, reverse primer in the seventh transmembrane domain. The single 780 bp fragment from both rat hippocampus and rat cerebellum were isolated from a 1% agarose gel, purified

using a GENECLAN III kit (BIO 101, Vista, Calif.) and sequenced using AmpliTaq DNA Polymerase, FS (Perkin Elmer). The sequence was run on an ABI PRISM 377 DNA Sequencer and analyzed using the Wisconsin Package (GCG, Genetics Computer Group, Madison, Wis.). This sequence was used to design PCR primers for the rat GABA<sub>B</sub>R2 gene.

#### Construction and Screening of a Rat Hypothalamic cDNA Library

[0356] Poly A<sup>+</sup> RNA was purified from rat hypothalamic RNA (Clontech) using a FastTrack kit (Invitrogen, Corp.). DS-cDNA was synthesized from 5  $\mu$ g of poly A<sup>+</sup> RNA according to Gubler and Hoffman (1983) with minor modifications. The resulting cDNA was ligated to BstXI adaptors (Invitrogen, Corp.) and the excess adaptors removed by exclusion column chromatography. High molecular weight fractions of size-selected ds-cDNA were ligated in pEXJ.T7, an Okayama and Berg expression vector modified from pEXV (Miller and Germain, 1986) to contain BstXI, other additional restriction sites, and a T7 promoter. A total of 100,000 independent clones with a mean insert size of 3.7 kb were generated. The library was amplified on agar plates (Ampicillin selection) in 48 primary pools. Glycerol stocks of the primary pools screened for a rat GABA<sub>B</sub>R2 gene by PCR using BB265, a forward primer from the loop between transmembrane domains 3 and 4 from the sequence determined above and BB266, a reverse primer from the sixth transmembrane domain from the sequence determined above. The conditions for PCR were 1 min at 94° C., 4 min at 68° C. for 40 cycles, with a pre- and post-incubation of 5 min at 95° C. and 7 min at 68° C., respectively. To determine which pools had the largest inserts, positive pools were screened by PCR using the vector primers BB172 or BB173, and a gene-specific primer BB265 or BB266. One positive primary pool, I-47, was subdivided into 24 pools of 1000 clones, and grown in LB medium overnight. Two  $\mu$ L of cultures were screened by PCR using primers BB172 and BB266. One positive subpool, I-47-4 was subdivided into 10 pools of 200 clones and plated on agar plates (ampicillin selection). Colonies were transferred to nitrocellulose membranes (Schleicher and Schuell, Keene, N.H.), denatured in 0.4 N NaOH, 1.5 M NaCl, renatured in 1M Tris, 1.5 M NaCl, and UV cross-linked. Filters were hybridized overnight at 40° C. in a buffer containing 50 % formamide, 0.12 M Na<sub>2</sub>HPO<sub>4</sub> (pH7.2), 0.25M NaCl, 7% SDS, 25 mg/L ssDNA and 10<sup>6</sup> cpm/mL of a cDNA probe corresponding to transmembrane domains 1 to 7 of rat GABA<sub>B</sub>R2, labeled with [<sup>32</sup>P]dCTP (3000 Ci/mmol, NEN) using a random prime labeling kit (Boehringer Mannheim). Filters were washed 1x5 min then 2x20 min at room temperature in 2xSSC, 0.1% SDS then 3x20 min at 500 in 0.1xSSC, 0.1% SDS and exposed to Biomax MS film (Kodak) for 3 hours. Four closely clustering colonies which appeared to hybridize were re-screened individually by PCR using primers BB265 and BB266, primers BB265 and BB55, primers BB265 and BB56, and primers BB266 and BB55. The conditions for PCR were 30 sec at 94° C., 2.5 min at 68° C. for 32 cycles, with a pre- and post-incubation of 5 min at 95° C. and 5 min at 68° C. respectively. One positive colony, I-47-4-2, was amplified overnight in 10 mL TB media and processed for

plasmid purification using a standard alkaline lysis miniprep procedure followed by a PEG precipitation. This plasmid was designated BO54 and partially sequenced using AmpliTaq DNA Polymerase, FS (Perkin Elmer). The sequence was run on an ABI PRISM 377 DNA Sequencer and analyzed using the Wisconsin Package (GCG, Genetics Computer Group, Madison, Wis.). BO54 was in the wrong orientation for expression in mammalian cells. To obtain a clone in the correct orientation, an EcoRI restriction fragment from BO54 was subcloned into the vector pEXJ. Transformants were screened by PCR using the primers BB56 and BB268 under the following conditions: 30 sec at 94° C., 2.5 min at 68° C. for 32 cycles, with a pre- and post-incubation of 5 min at 95° C. and 3 min at 68° C. respectively. One transformant in the correct orientation was amplified overnight in 100 ml

[0357] TB media and processed for plasmid purification using a standard alkaline lysis miniprep procedure followed by a PEG precipitation. This plasmid was designated BO55 and sequenced using AmpliTaq DNA Polymerase, FS (Perkin Elmer). Plasmid BO-55 was deposited with the ATCC on Jun. 10, 1997, and was accorded ATCC Accession No. 209104. The sequence of BO-55 was determined using an ABI PRISM 377 DNA Sequencer and analyzed using the Wisconsin Package (GCG, Genetics Computer Group, Madison, Wis.).

#### Primers Used

[0358]

BB257: 5'-CTCTCTGCCCTCACCATCCTCGGGAT-3' (SEQ ID NO:21)

BB258: 5'-GACTCCGGCTCGAATACCAGGCAGAG-3' (SEQ ID NO:22)

BB265: 5'-CCATGTTTGCAAAGACCTGGAGGGTCC-3' (SEQ ID NO:23)

BB266: 5'-GGTCACGCGTCAGGAAAGACAGCAG-3' (SEQ ID NO:24)

BB172: 5'-AAGCTTCTAGAGATCCCTCGACCTC-3' (SEQ ID NO:25)

BB173: 5'-AGGCGCAGAACTGGTAGGTATGGAA-3' (SEQ ID NO:26)

BB55: 5'-CTTCTAGGCCCTGTACGGAAGTGTTA-3' (SEQ ID NO:27)

BB56: 5'-GTTGTGGTTTGTCCAACTCATCAATG-3' (SEQ ID NO:28)

BB268: 5'-CTGCTGTCTCTTTCCTGACGCGTGACC-3' (SEQ ID NO:29).

#### Generation of DNA Coding for Rat GABA<sub>B</sub>1b and GABA<sub>B</sub>1a Polypeptides

[0359] The gene encoding the rat GABA<sub>B</sub>R1b polypeptide was obtained by screening the same rat hypothalamic library used for GABA<sub>B</sub>R2 with primers based on the original publication of the clone by Kaupmann, et al., 1997. A partial clone lacking the first 55 nucleotides was identified and ligated to a PCR fragment containing the missing base pairs to obtain the full length clone. A restriction fragment containing the entire coding region of GABA<sub>B</sub>R1b was subcloned into the mammalian expression vector pEXJ.T7 and designated "BO58". A rat GABA<sub>B</sub>1a polypeptide clone was

obtained by ligating a restriction fragment of the GABA<sub>B</sub>1b clone, which contained the common region of the GABA<sub>B</sub>1 gene, to a PCR product containing the GABA<sub>B</sub>1a-specific 5' end.

#### In Situ Hybridization Experiments for GABA<sub>B</sub>R2 mRNA Animals

[0360] Male Sprague-Dawley rats (Charles Rivers, Rochester, N.Y.) were euthanized using CO<sub>2</sub>, decapitated, and their brains immediately removed and rapidly frozen on crushed dry ice. Coronal sections of brain tissue were cut at 11  $\mu$ m using a cryostat and thaw-mounted onto poly-L-lysine-coated slides and stored at -20° C. until use.

#### Tissue Preparation

[0361] Prior to hybridization, the tissues were fixed in 4% paraformaldehyde/PBS pH 7.4 followed by two washes in PBS (Specialty Media, Lavallete, N.J.). Tissues were then treated in 5 mM dithiothreitol, rinsed in DEPC-treated PBS; acetylated in 0.1 M triethanolamine containing 0.25% acetic anhydride, rinsed twice in 2 $\times$ SSC, delipidated with chloroform then dehydrated through a series of graded alcohols. All reagents were purchased from Sigma (St. Louis, Mo.).

#### Radioactive In Situ Hybridization Histochemistry

[0362] Oligonucleotide probes, MJ79/80, corresponding to nucleotides 354-398 and MJ109/110, corresponding to

nucleotides 952-991 of the rat GABA<sub>B</sub>R2 cDNA, MJ94/95, corresponding to nucleotides 151-193 of the human GABA<sub>B</sub>R1a cDNA, and MJ83/84, corresponding to nucleotides 34-71 of the rat GABA<sub>B</sub>R1b cDNA were used to characterize the distribution of each polypeptides' respective mRNA. The oligonucleotides were synthesized using an Expedite Nucleic Acid Synthesis System (PerSeptive Biosystems, Framingham, Mass.) and purified using 12% polyacrylamide gel electrophoresis. Additionally, sense and antisense oligonucleotides corresponding to positions 1076-1120 of GABA<sub>B</sub>R1b (1424-1468 of GABA<sub>B</sub>R1a) were used (BB403 and BB404).

[0363] The sequences of the oligonucleotides are:

[0364] For rat GABA<sub>B</sub>R2:

Sense probe, MJ79:  
5' - GCA ATA AAG TAT GGG CTG AAC CAT TTG (SEQ ID NO:36)  
ATG GTG TTT GGA GGC GT - 3'

Antisense probe, MJ80:  
5' - ACG CCT CCA AAC ACC ATC AAA TGG TTC (SEQ ID NO:37)  
AGC CCA TAC TTT ATT GC - 3'

Sense probe, MJ109:  
5' - TTT GAG CCC CTG AGC TCC AAA CAA ATC (SEQ ID NO:38)  
AAG ACC ATC TCA G - 3'

Antisense probe, MJ110:  
5' - CTG AGA TGG TCT TGA TTT GTT TGG AGC (SEQ ID NO:39)  
TCA GGG GCT CAA A - 3'

For human GABA<sub>B</sub>R1a:  
Sense probe, MJ94:  
5' - AAG GCC ATC AAC TTC CTG CCT GTG GAC (SEQ ID NO:40)  
TAT GAG ATC GAA TAT G - 3'

Antisense probe, MJ95:  
5' - CAT ATT CGA TCT CAT AGT CCA CAG GCA (SEQ ID NO:41)  
GGA AGT TGA TGG CCT T - 3'

For rat GABA<sub>B</sub>R1b:  
Sense probe, MJ83:  
5' - TGG CCG CTG CCT CTT CTG CTG GTG ATG (SEQ ID NO:42)  
GCG GCT GGG GT - 3'

Antisense probe, MJ84:  
5' - ACC CCA GCC GCC ATC ACC AGC AGA AGA (SEQ ID NO:43)  
GGC AGC GGC CA - 3'

Sense probe, BB403:  
5' - CCT TGG CTT TGG CCT TGA ACA AGA CGT (SEQ ID NO:44)  
CTG GAG GAG GTG GTC GTT - 3'

Antisense probe, BB404:  
5' - AAC GAC CAC CTC CTC CAG ACG TCT TGT (SEQ ID NO:45)  
TCA AGG CCA AAG CCA AGG - 3'

[0365] Probes were 3'-end labeled with [<sup>35</sup>S]dATP (1200Ci/mmol, NEN, Boston, Mass.) to a specific activity of 109 dpm/pg using terminal deoxynucleotidyl transferase (Pharmacia, Piscataway, N.J.). In situ hybridization was done with modification of the method described by Durkin, M, et al, 1995.

#### Nonradioactive In Situ Hybridization Histochemistry

[0366] Antisense/sense probes corresponding to nucleotides 354-398 of the rat GABA<sub>B</sub>R2 cDNA, were 3'-end labeled with digoxigenin using TdT. The labeling reaction was carried out as outlined in the DIG/Genius System, (Boehringer Mannheim, Indianapolis, Ind.). Conditions used in ISHH with digoxigenin-labeled probes are the same as described above. The sections were rinsed in buffer 1, washing buffer (0.1 M Tris-HCl pH 7.5/0.15 M NaCl), pre-incubated in Blocking Solution (Buffer 1, 0.1% Triton-X and 2% normal sheep serum) for 30 minutes and then incubated for 2 hours in Blocking Solution containing anti-digoxigenin-AP Fab fragment (Boehringer Mannheim) at 1:500 dilution followed by two 10 minute washes in Buffer 1. To develop color, sections were rinsed in Detection Buffer (0.1M Tris-HCl pH 9.5/0.15M NaCl/0.05 M MgCl<sub>2</sub>) for 10 minutes and then incubated overnight in Detection Buffer containing 0.5 mM NBT, 0.1 mM BCIP, and 1 mM levamisole. After color development, slides were dipped in dH<sub>2</sub>O and coverslipped using aqua mount.

[0367] Probe specificity was established by performing in situ hybridization on HEK293 cells transiently transfected with eukaryotic expression vectors containing the rat GABA<sub>B</sub>R1b and human GABA<sub>B</sub>R1a DNA or no insert for transfection. Furthermore, two pairs of hybridization probes, sense and antisense, that were targeted to different segments of the GABA<sub>B</sub>R2 mRNA were used for cells and rat tissues.

#### Quantification

[0368] The strength of the hybridization signal obtained in various region of the rat brain was graded as weak (+), moderate (++), heavy (+++) or intense (++++). These were qualitative evaluations for each of the polypeptide mRNA distributions based on the relative optical density on the autoradiographic film and on the relative number of silver grains observed over individual cells at the microscopic level.

#### Cell Culture

[0369] COS-7 cells are grown on 150 mm plates in DMEM with supplements (Dulbecco's Modified Eagle Medium with 10% bovine calf serum, 4 mM glutamine, 100 units/mL penicillin/100 Ag/mL streptomycin) at 37° C., 5% CO<sub>2</sub>. Stock plates of COS-7 cells are trypsinized and split 1:6 every 3-4 days.

[0370] Human embryonic kidney 293 cells are grown on 150 mm plates in DMEM with supplements (10% bovine calf serum, 4 mM glutamine, 100 units/mL penicillin/100

$\mu\text{g/mL}$  streptomycin) at 37° C., 5% CO<sub>2</sub>. Stock plates of 293 cells are trypsinized and split 1:6 every 3-4 days.

[0371] Mouse fibroblast LM(tk-) cells are grown on 150 mm plates in D-MEM with supplements (Dulbecco's Modified Eagle Medium with 10% bovine calf serum, 4 mM glutamine, 100 units/mL penicillin/100  $\mu\text{g/mL}$  streptomycin) at 37° C., 5% CO<sub>2</sub>. Stock plates of LM(tk-) cells are trypsinized and split 1:10 every 3-4 days.

[0372] Chinese hamster ovary (CHO) cells are grown on 150 mm plates in HAM's F-12 medium with supplements (10% bovine calf serum, 4 mM L-glutamine and 100 units/mL penicillin/100  $\mu\text{g/mL}$  streptomycin) at 37° C., 5% CO<sub>2</sub>. Stock plates of CHO cells are trypsinized and split 1:8 every 3-4 days.

[0373] Mouse embryonic fibroblast NIH-3T3 cells are grown on 150 mm plates in Dulbecco's Modified Eagle Medium (DMEM) with supplements (10% bovine calf serum, 4 mM glutamine, 100 units/mL penicillin/100  $\mu\text{g/mL}$  streptomycin) at 37° C., 5% CO<sub>2</sub>. Stock plates of NIH-3T3 cells are trypsinized and split 1:15 every 3-4 days.

[0374] Sf9 and Sf21 cells are grown in monolayers on 150 mm tissue culture dishes in TMN-FH media supplemented with 10% fetal calf serum, at 27° C., no CO<sub>2</sub>. High Five insect cells are grown on 150 mm tissue culture dishes in Ex-Cell 400™ medium supplemented with L-Glutamine, also at 27° C., no CO<sub>2</sub>.

[0375] LM(tk-) cells stably transfected with the DNA encoding the polypeptides disclosed herein may be routinely converted from an adherent monolayer to a viable suspension. Adherent cells are harvested with trypsin at the point of confluence, resuspended in a minimal volume of complete DMEM for a cell count, and further diluted to a concentration of 10<sup>6</sup> cells/mL in suspension media (10% bovine calf serum, 10% 10× Medium 199 (Gibco), 9 mM NaHCO<sub>3</sub>, 25 mM glucose, 2 mM L-glutamine, 100 units/mL penicillin/100  $\mu\text{g/mL}$  streptomycin, and 0.05% methyl cellulose). Cell suspensions are maintained in a shaking incubator at 37° C., 5% CO<sub>2</sub> for 24 hours. Membranes harvested from cells grown in this manner may be stored as large, uniform batches in liquid nitrogen.

[0376] Alternatively, cells may be returned to adherent cell culture in complete DMEM by distribution into 96-well microtiter plates coated with poly-D-lysine (0.01 mg/mL) followed by incubation at 37° C., 5% CO<sub>2</sub> for 24 hours.

#### Generation of Baculovirus

[0377] The coding region of DNA encoding the polypeptides disclosed herein may be subcloned into pBlueBacIII into existing restriction sites, or sites engineered into sequences 5' and 3' to the coding region of the polypeptides. To generate baculovirus, 0.5  $\mu\text{g}$  of viral DNA (BaculoGold) and 3  $\mu\text{g}$  of DNA construct encoding a polypeptide may be co-transfected into 2×10<sup>6</sup> *Spodoptera frugiperda* insect Sf9 cells by the calcium phosphate co-precipitation method, as outlined in by Pharmingen (in "Baculovirus Expression Vector System: Procedures and Methods Manual") The cells then are incubated for 5 days at 27° C.

[0378] The supernatant of the co-transfection plate may be collected by centrifugation and the recombinant virus plaque

purified. The procedure to infect cells with virus, to prepare stocks of virus and to titer the virus stocks are as described in Pharmingen's manual.

#### Transfection

[0379] All subtypes studied may be transiently transfected into COS-7 cells by the DEAE-dextran method, using 1  $\mu\text{g}$  of DNA/10<sup>6</sup> cells (Cullen, 1987). In addition, Schneider 2 *Drosophila* cells may be cotransfected with vectors containing the gene, under control of a promoter which is active in insect cells, and a selectable resistance gene, eg., the G418 resistant neomycin gene, for expression of the polypeptides disclosed herein.

#### Stable Transfection

[0380] DNA encoding the polypeptides disclosed herein may be co-transfected with a G-418 resistant gene into the human embryonic kidney 293 cell line by a calcium phosphate transfection method (Cullen, 1987). Stably transfected cells are selected with G-418.

#### Radioligand Binding Assays

[0381] Transfected cells from culture flasks were scraped into 5 mL of Tris-HCl, 5 mM EDTA, pH 7.5, and lysed by sonication. The cell lysates were centrifuged at 1000 rpm for 5 min. at 4° C., and the supernatant was centrifuged at 30,000×g for 20 min. at 4° C. The pellet was suspended in binding buffer (50 mM Tris-HCl, 2.5 mM CaCl<sub>2</sub> at pH 7.5 supplemented with 0.1% BSA, 2  $\mu\text{g/mL}$  aprotinin, 0.5 mg/mL leupeptin, and 10  $\mu\text{g/mL}$  phosphoramidon). Optimal membrane suspension dilutions, defined as the protein concentration required to bind less than 10% of the added labeled compound (typically a radiolabeled compound), were added to 96-well polypropylene microtiter plates containing labeled compound, unlabeled compounds (i.e., displacing ligand in an equilibrium competition binding assay) and binding buffer to a final volume of 250  $\mu\text{L}$ . In equilibrium saturation binding assays membrane preparations were incubated in the presence of increasing concentrations of labeled compound. The binding affinities of the different compounds were determined in equilibrium competition binding assays, using labeled compound, such as 1 nM [<sup>3</sup>H]-CGP54626, in the presence of ten to twelve different concentrations of the displacing ligand(s). Some examples of displacing ligands included GABA, baclofen, 3APMPA, phaclofen, CGP54626, and CGP55845. Mixtures of several unlabeled test compounds (up to about 10 compounds) may also be used in competition binding assays, to determine whether one of the mixture component compounds binds to the polypeptide or receptor. Binding reaction mixtures were incubated for 1 hr at 30° C., and the reaction was stopped by filtration through GF/B filters treated with 0.5% polyethyleneimine, using a cell harvester. Where the labeled compound was a radiolabeled compound, the amount of bound compound was evaluated by gamma counting (for <sup>125</sup>I) or scintillation counting (for <sup>3</sup>H). Data were analyzed by a computerized non-linear regression program. Non-specific binding was defined as the amount of radioactivity remaining after incubation of membrane protein in the presence of excess unlabeled compound. Protein concentration may be measured by the Bradford method using Bio-Rad Reagent, with bovine serum albumin as a standard.

### Cyclic AMP (cAMP) Formation Assay

[0382] The receptor-mediated inhibition of cyclic AMP (cAMP) formation may be assayed in transfected cells expressing the mammalian receptors described herein. Cells are plated in 96-well plates and incubated in Dulbecco's phosphate buffered saline (PBS) supplemented with 10 mM HEPES, 5 mM theophylline, 2  $\mu$ g/ml aprotinin, 0.5 mg/ml leupeptin, and 10  $\mu$ g/ml phosphoramidon for 20 min at 37° C., in 5% CO<sub>2</sub>. Test compounds are added and incubated for an additional 10 min at 37° C. The medium is then aspirated and the reaction stopped by the addition of 100 mM HCl. The plates are stored at 4° C. for 15 min, and the cAMP content in the stopping solution measured by radioimmunoassay. Radioactivity may be quantified using a gamma counter equipped with data reduction software.

### Generation of Chimeric G-proteins

[0383] Chimeric G-proteins were constructed using standard mutagenesis methods (Conklin et al., 1993). Two chimeras were constructed. The first comprises the entire coding region of human G $\alpha_q$  with the exception of the final 3' 15 nucleotides which encode the C-terminal 5 amino acids of G $\alpha_{13}$ . The second also comprises the entire coding region of human G $\alpha_q$  with the exception of the final 3' 15 nucleotides, which encode the C-terminal 5 amino acids of G $\alpha_z$ . Sequences of both chimeric G-protein genes were verified by nucleotide sequencing. For the purposes of expression in oocytes, synthetic mRNA transcripts of each gene were synthesized using the T7 polymerase.

### Phosphoinositide Assay

[0384] The agonist activities of GABA-B agonists were assayed by measuring their ability to generate phosphoinositide production in COS-7 cells transfected transiently with GABA<sub>B</sub>R1, GABA<sub>B</sub>R2, and chimeric G $\alpha_{q/z}$ . Alternatively, COS-7 cells are transfected transiently with GABA<sub>B</sub>R1, GABA<sub>B</sub>R2, and other chimeric G-protein alpha subunits such as G $\alpha_{q/12}$ , G $\alpha_{q/13}$ , or G $\alpha_{q/10}$ . Cells were plated in 96-well plates and grown to confluence. The day before the assay the growth medium was changed to 100  $\mu$ l of medium containing 1% serum and 0.5  $\mu$ Ci [<sup>3</sup>H]myo-inositol, and the plates were incubated overnight in a CO<sub>2</sub> incubator (5% CO<sub>2</sub> at 37° C.).

[0385] Immediately before the assay, the medium was removed and replaced by 200  $\mu$ l of PBS containing 10 mM

plate row. All assays were performed in duplicate by repeating the same additions in two consecutive rows. The plates were incubated in a CO<sub>2</sub> incubator for 30 min. The reaction was terminated by removal of the buffer solution by blotting, followed by the addition of 100  $\mu$ l of 50% (v/v) trichloroacetic acid (TCA), and 10 min incubation at 4° C.

[0386] The contents of the wells were then transferred to a Multiscreen HV filter plate (Millipore) containing Dowex AG1-X8 (200-400 mesh, formate form). The filter plates were prepared adding 100  $\mu$ l of Dowex AG1-X8 suspension (50% v/v, water:resin) to each well. The filter plates were placed on a vacuum manifold to wash or elute the resin bed. Each well was washed 3 times with 200  $\mu$ l of 5 mM myo-inositol. The [<sup>3</sup>H]-IPs were eluted into empty 96-well plates with 75  $\mu$ l of 1.2 M ammonium formate/0.1 M formic acid. After the addition of 200  $\mu$ l of scintillation cocktail (Optiphase Supermix; Wallac) to each well, [<sup>3</sup>H]-IPs were quantified by counting on a Trilux 1450 Microbeta scintillation counter.

### Oocyte Expression

[0387] Female *Xenopus laevis* (Xenopus-1, Ann Arbor, Mich.) are anesthetized in 0.2% tricain (3-aminobenzoic acid ethyl ester, Sigma Chemical Corp.) and a portion of ovary is removed using aseptic technique (Quick and Lester, 1994). Oocytes are defolliculated using 3 mg/ml collagenase (Worthington Biochemical Corp., Freehold, N.J.) in a solution containing 87.5 mM NaCl, 2 mM KCl, 2 mM MgCl<sub>2</sub> and 5 mM HEPES, pH 7.5. Oocytes are injected (Nanoject, Drummond Scientific, Broomall, Pa.) with 50-70 nl mRNA prepared as described below. After injection of mRNA, oocytes are incubated at 17 degrees for 3-8 days.

[0388] RNAs are prepared by transcription from: (1), linearized DNA plasmids containing the complete coding region of the gene, or (2), templates generated by PCR incorporating a T7 promoter and a poly A<sup>+</sup> tail. From either source, DNA is transcribed into mRNA using the T7 polymerase ("Message Machine", Ambion).

[0389] The transcription template for the rat GABA<sub>B</sub>R1b gene was prepared by PCR amplification of the plasmid BO58 using the primers MJ23 and MJ47 (see below). The template for the rat GABA<sub>B</sub>R2 gene was made by linearization of the plasmid BO56, rat GABA<sub>B</sub>R2 insert from BO55 in the expression vector pEXJ.T7, with NotI.

[0390] Primers:

MJ23 5' CCAAGCTTCTAATACGACTCACTATAGGGGAGACCATGGGCCCGGGGGG (SEQ ID NO:30);  
 ACCCTGTACC 3'  
 MJ47 5' T<sub>(35)</sub>CACCTGTAAAGCAAAATGTACTCGACTCC 3' (SEQ ID NO:31).

LiCl, and the cells were equilibrated with the new medium for 20 min. The [<sup>3</sup>H]inositol-phosphate (IP) accumulation was started by adding 22  $\mu$ l of a solution containing the agonist. To the first two wells 22  $\mu$ l of PBS were added to measure basal accumulation, and 10 different concentrations of agonist were assayed in the following 10 wells of each

[0391] Genes encoding G-protein inwardly rectifying K<sup>+</sup> channels 1 and 4 (GIRK1 and GIRK4; "GIRKs") were obtained by PCR using the published sequences (Kubo et al., 1993; Dascal et al., 1993; Krapivinsky et al., 1995b) to derive appropriate 5' and 3' primers. Human heart cDNA was used as template together with the primers



5' -CGCGGATCCATTATGTCGCTCCGAGGAAATTG-3' (SEQ ID NO:32) and  
 5' -CGCGAATTCCTATGTGAAGCGATCAGAGTTCATTTTC-3' (SEQ ID NO:33) for GIRK1  
 and  
 5' -GCGGGATCCGCTATGCTGGTGATCTAGGAATG-3' (SEQ ID NO:34) and  
 5' -CCGGAATTCCTCCCTCACACCGAGCCCTGG-3' (SEQ ID NO:35) for GIRK4.

[0392] The BamHI and EcoRI restriction sites in each primer pair were used to clone the PCR product into the expression vector pcDNA-Amp (Invitrogen). Plasmid vectors containing GIRK1 and GIRK4 are referred to as "JS1800" and "JS1741", respectively. The coding regions of both genes were sequenced and verified.

#### Oocyte Electrophysiology

[0393] Dual electrode voltage clamp ("GeneClamp", Axon Instruments Inc., Foster City, Calif.) is performed using 3 M KCl-filled glass microelectrodes having resistances of 1-3 Mohms. Unless otherwise specified, oocytes are voltage clamped at a holding potential of -80 mV. During recordings, oocytes are bathed in continuously flowing (1-3 ml/min) medium containing 96 mM NaCl, 2 mM KCl, 1.8 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, and 5 mM HEPES, pH 7.5 (ND96), or elevated K<sup>+</sup> containing 49 mM KCl, 49 mM NaCl, 1.8 mM CaCl<sub>2</sub>, 2 mM MgCl<sub>2</sub>, and 5 mM HEPES, pH 7.5 (hK). Drugs are applied either by local perfusion from a 10  $\mu$ l glass capillary tube fixed at a distance of 0.5 mm from the oocyte, or for calculation of steady-state EC<sub>50</sub>s, by switching from a series of gravity fed perfusion lines. Experiments are carried out at room temperature. All values are expressed as mean  $\pm$  standard error of the mean.

[0394] Concentration-response curves for agonists and antagonists were fitted with logistic equations of the form  $I=1/(1+(EC_{50}/[Agonist])^n)$  for agonists and  $I=1/(1+([Antagonist]/IC_{50})^n)$  for antagonists, where I is current, where EC<sub>50</sub> is the concentration of agonist that produced half-maximal activation, IC<sub>50</sub> is the concentration of antagonist that produced half-maximal inhibition, and n the Hill coefficient. Fits were made with a Marquardt-Levenberg non-linear least-squares curve fitting algorithm.

#### Recording Ion Currents in Mammalian Cells

[0395] The ability of the rat GABA<sub>B</sub>R1 and GABA<sub>B</sub>R2 genes to activate GIRK currents in mammalian cells was investigated by transient transfection of HEK-293 cells followed by voltage clamp analysis of currents. HEK-293 cells were maintained in Dulbecco's modified Eagle medium (DMEM) plus 10% (v/v) bovine calf serum, 2% L-glutamine, 50 U/ml penicillin, and 50  $\mu$ g/ml streptomycin and were incubated at 37° C. in a humidified 5% CO<sub>2</sub> atmosphere. Cells were harvested twice each week by treatment with 0.25% trypsin/1 mM EDTA in Hank's Salts and re-seeded at 20% of their original density either into 75 cm<sup>2</sup> flasks (for passaging) or into 35 mm tissue culture dishes (for transfection and electrophysiology experiments).

[0396] HEK-293 cells, 40%-80% confluent, were co-transfected with various combinations of 0.6  $\mu$ g each of the following plasmids: pGreen Lantern-1 (Gibco/BRL, Gaithersburg, Md.), human GIRK1 (JS1800), human GIRK4 (JS1741), rat GABA<sub>B</sub>R1b (BO58), and rat

GABA<sub>B</sub>R2 (BO55). Cells were transiently transfected using the Superfect Transfection Reagent from Qiagen (Valencia, Calif.) according to the manufacturer's instructions. Briefly, 3  $\mu$ g total plasmid DNA were incubated with 22.5  $\mu$ l Superfect Reagent in 100  $\mu$ l serum-free DMEM for 5-10 minutes at room temperature. After addition of 600  $\mu$ l complete DMEM, the DNA/Superfect mixture was transferred to cells growing in 35 mm dishes coated with poly-D-lysine and incubated for 2-4 hours at 37° C. in a 5% CO<sub>2</sub> incubator. Subsequently, the dishes were washed once with phosphate-buffered saline and 2 ml complete DMEM was added. Cells were incubated for 24-72 hours at 37° C. before performing electrophysiological measurements.

[0397] The whole-cell configuration of the patch-clamp technique was used with glass pipettes having resistances of 2-4 M $\Omega$  when filled with the pipette solution. Solutions used were (in mM), KMeSO<sub>4</sub>, 125; KCl, 5; NaCl, 5; MgCl<sub>2</sub>, 2; EGTA, 11; HEPES, 10, pH 7.4; MgATP, 1.0; Na<sub>2</sub>GTP, 0.2, for the pipette and NaCl, 130; KCl, 4; CaCl<sub>2</sub>, 2; MgCl<sub>2</sub>, 2; Glucose, 10; Sucrose, 10; HEPES, 10, pH 7.4 for the bath. GIRK currents were recorded in elevated K<sup>+</sup> solution containing 25 mM K<sup>+</sup> and a correspondingly lower concentration of Na<sup>+</sup>. Voltage clamp recordings were made with an EPC-9 amplifier using Pulse+PulseFit software (HEKA Elektronik). Series resistances were kept below 10 Mohm and no attempt was made to provide series resistance compensation. Currents were low-pass filtered at 1 kHz and digitized at a rate of 5 kHz. Unless otherwise noted, experiments were performed at room temperature on cells voltage clamped at a holding potential of -70 mV. Application of agonists was realized using a gravity-fed, perfusion system consisting of six concentrically arranged microcapillary tubes (Jones et al. 1997). The time to complete solution exchange was about 100 ms. The bath was constantly perfused at a low rate with control solution.

[0398] All voltage clamp recordings were made from transfected cells visualized under epifluorescent lighting conditions utilizing a filter set designed for GFP (Zeiss Optics). Fluorescent cells were an excellent indication of transfection since they all exhibited some constitutive GIRK current activity in contrast to untransfected cells which displayed no measurable inward rectifier K<sup>+</sup> currents (data not shown).

#### Microphysiometry

[0399] GABA<sub>B</sub>R1, GABA<sub>B</sub>R2 or the combination, were transiently expressed in CHO-K1 cells by liposome mediated transfection according to the manufacturer's recommendations ("LipofectAMINE", GibcoBRL, Bethesda, Md.), and maintained in Ham's F-12 medium with 10% bovine serum. Cells were prepared for microphysiometric recording as previously described (Salon, J. A., et al., 1995). On the day of the experiment the cell capsules were trans-

ferred to the microphysiometer and allowed to equilibrate in recording media (low buffer RPMI 1640; no bicarbonate, no serum, Molecular Devices Corp.), during which a baseline was established. The recording paradigm consisted of a 100 ml/min flow rate and a 30 s flow interruption during which the rate measurement was taken. Challenges involved an 80 s drug exposure just prior to the first post-challenge rate measurement being taken, followed by two additional pump cycles. Acidification rates reported are expressed as a percentage increase of the peak response over the baseline rate observed just prior to challenge.

#### N-terminal Deletion Experiments

[0400] As a start to exploring the structural aspects of GABA<sub>B</sub>R2 important for functional activity of the GABA<sub>B</sub>R1/R2 receptor, N-terminal deletion experiments were performed on the GABA<sub>B</sub>R2-HA construct (see below). All such deletion mutants caused a complete disruption of receptor activity as assessed by the measurement of GIRK currents in transfected HEK293 cells. In one such experiment, wildtype GABA<sub>B</sub>R2-HA was digested with BglII restriction enzyme and religated. The BglII deletion mutant (M118) lacks 257 amino acids at the N-terminus, corresponding to positions 226-482. Using immunofluorescence, M118 was found to be expressed on the cell surface, similarly to the wildtype GABA<sub>B</sub>R2-HA, yet when co-expressed with GABA<sub>B</sub>R1 did not produce GIRK activation with 100  $\mu$ M GABA. Thus, although we cannot yet identify specific amino acids contributing to receptor activity, it appears that the N-terminal region comprising amino acids 226-482 is critically important either for dimer formation, ligand binding or conformational changes associated with signal transduction.

#### Construction of Epitope-tagged Polypeptides and Confocal Microscopy

[0401] Incorporation of sequences encoding the RGS6xHis or influenza virus hemagglutinin (HA) epitope into the GABA<sub>B</sub>R1 and GABA<sub>B</sub>R2 genes, respectively, was performed by PCR. Each epitope was positioned immediately before the stop codon in the appropriate gene. Both tagged genes were subcloned into pcDNA. Sequence analysis was used to confirm all PCR-derived portions of the construct. Forty-eight hours post-transfection HEK293 cells were fixed for 20 min in 4% paraformaldehyde in PBS, permeabilized in PBS containing 2% BSA and 0.1% Triton X-100 and incubated with primary antibody for 1.5 h. Mouse monoclonal anti-RGS (Qiagen) and mouse anti-FLAG (Boehringer-Mannheim) were labeled with FITC-conjugated goat anti-mouse antibodies. Rat monoclonal anti-HA (Boehringer-Mannheim) was visualized with TRITC-conjugated rabbit anti-rat antibodies. Fluorescent images were obtained with a Zeiss LSM 410 confocal microscope using a 100 $\times$  oil-immersion objective.

#### Immunoprecipitation and Western Blotting

[0402] Forty-eight hours following transient transfection HEK293 cells were solubilized in lysis buffer containing (in mM): 50 Tris/Cl pH 7.4, 300 NaCl, 1.5 MgCl<sub>2</sub>, 1 CaCl<sub>2</sub>, protease inhibitors (Boehringer Mannheim tablets), 1% Triton X-100, and 10% glycerol. 1-2 mg of protein was immunoprecipitated overnight at 4 $^{\circ}$  C. with either 0.5  $\mu$ g rat monoclonal anti-HA antibody or 0.5  $\mu$ g mouse monoclonal

anti-4xHis antibody (Qiagen). Immune complexes were bound to 20  $\mu$ l Protein-A agarose (Research Diagnostics, Inc.) for 2 h at RT. Protein-A pellets were washed twice with buffer containing Triton-X-100, then once without, and eluted with 80  $\mu$ l Laemmli sample buffer containing 2% (w/v) SDS and 20 mM DTT. After heating for 3 min. at 70 $^{\circ}$  C., 20  $\mu$ l IP samples or 20  $\mu$ g total protein was subjected to SDS-PAGE followed by Western blotting with either anti-HA or anti-4xHis antibody, followed by sheep anti-rat (Amersham) or goat anti-mouse (RDI) HRP-linked secondary antibodies, respectively. Proteins were visualized with enhanced chemiluminescent substrates (Pierce).

[0403] Alternatively, material for immunoprecipitations was obtained by sucrose gradient fractionation of the P1 pellet as described by Graham (Graham, 1984). To verify the enrichment of plasma membrane in the resulting "P1+" pellet, Na<sup>+</sup>/K<sup>+</sup> ATPase in the P1+ and P2 (primarily microsomal and vesicular (Graham, 1984)) fractions was quantified by fluorescence detection of anti-alpha 1 subunit antibody (Research Diagnostics, Inc., clone 9A-5) on a phosphor imager (Molecular Dynamics). ATPase in P1+ fractions used for immunoprecipitations was found to be enriched >50 fold compared to P2 fractions.

#### Experimental Results

##### Novel GPCR Sequences Identified by BLAST Search

[0404] The rat GABA<sub>B</sub>R1a amino acid sequence (Kaupmann et al. (1997) Nature 386:239) was used as a query to search the EST division of GenBank with BLAST. Two entries, T07621 and Z43654, had probability scores that suggested significant amino acid homology to the GABA<sub>B</sub>R1a polypeptide. T07621 had sequence homology from the beginning of the first transmembrane domain to the beginning of third transmembrane domain of the GABA<sub>B</sub>R1a polypeptide. Z43654 had sequence homology from the sixth transmembrane domain to the seventh transmembrane domain of the GABA<sub>B</sub>R1a polypeptide. The sequence documentation for T07621 and Z43654 was retrieved with Entrez (NCBI) and neither sequence was annotated as having homology to any 7-TM spanning protein.

[0405] These results were used to obtain a full-length human clone TL-266, comprising both of the sequences identified by the BLAST search. Sequence analysis of clone TL-266 revealed a complete coding region for a novel protein. A search of the GenEMBL database indicated that the most similar sequence was that of GABA<sub>B</sub>R1a, followed by G protein-coupled receptors (GPCRs) of the metabotropic receptor superfamily. The nucleotide and deduced amino acid sequence of TL-266 are shown in FIGS. 1 and 2, respectively. The nucleotide sequence of the coding region is 57% identical to the rat GABA<sub>B</sub>R1a over a region of 1,686 bases. The longest open reading frame encodes an 898 amino acid protein with 38% amino acid identity to the rat GABA<sub>B</sub>R1a polypeptide. Hydrophathy plots of the predicted amino acid sequence reveal seven hydrophobic regions that may represent transmembrane domains (TMs, data not shown), typical of the G protein-coupled receptor superfamily. In the putative TM domains, GABA<sub>B</sub>R2 exhibits 45% amino acid identity with the rat GABA<sub>B</sub>R1a polypeptide. The amino terminus of TL-266

has amino acid homology to the bacterial periplasmic binding protein, a common feature of the metabotropic receptors (O'Hara et al. (1993) *Neuron* 11:41-52).

#### Generation of rat GABA<sub>B</sub>R2 PCR Product

[0406] Using PCR primers designed against the first and seventh transmembrane domains of the human GABA<sub>B</sub>R2 sequence, BB257 and BB258, a 780 base pair fragment was amplified from rat hippocampus and rat cerebellum. Sequence from these bands displayed 90% nucleotide identity to the human GABA<sub>B</sub>R2 gene. This level of homology is typical of a species homologue relationship in the GPCR superfamily.

#### Construction and Screening of a Rat Hypothalamic cDNA Library

[0407] To obtain a full-length rat GABA<sub>B</sub>R2 clone, pools of a rat hypothalamic cDNA library were screened by PCR using primers BB265 and BB266. A 440 base pair fragment was amplified from 44 out of 47 pools. Vector-anchored PCR was performed to identify pools with the largest insert size. One positive primary pool, I-47, was subdivided into 24 pools of 1000 individual clones and screened by vector-anchored PCR. Seven positive subpools were identified and one, I-47-4, was subdivided into 10 pools of 200 clones, plated onto agar plates, and screened by southern analysis. Four closely clustering colonies that appeared positive were rescreened individually by vector-anchored PCR. One positive colony, I-47-4-2, designated BO54, was amplified as a single rat GABA<sub>B</sub>R2 clone. Since vector-anchored PCR revealed that BO54 was in the wrong orientation for expression, the insert was isolated by restriction digest and subcloned into the expression vector pEX1. A transformant in the correct orientation was identified by vector-anchored PCR, and designated BO-55.

[0408] The nucleotide and deduced amino acid sequence of BO-55 are shown in FIGS. 3 and 4, respectively. BO-55 contains a 2.82 kD open reading frame and encodes a polypeptide of 940 amino acids. The nucleotide sequence of BO-55 is 89% identical to TL-267 in the coding region, with an overall amino acid identity of 98%. The proposed signal peptide cleavage site is between amino acids 40 and 41 (Nielsen et al., 1997).

[0409] A BLAST search of GenEMBL indicated that this sequence was most closely related to GABA<sub>B</sub>R1, displaying 35% and 41% amino acid identities overall and within the predicted transmembrane domains, respectively (FIG. 10). The structural similarity to GABA<sub>B</sub>R1 indicated that this sequence encodes a novel polypeptide, which we refer to as GABA<sub>B</sub>R2. The next most related sequences were other members of the mGluR family, with 21-24% overall amino acid identities. Like GABA<sub>B</sub>R1 and other members of the mGluR family (O'Hara, P. J., et al., 1998), GABA<sub>B</sub>R2 contains a large N-terminal extracellular domain having regions of homology to bacterial periplasmic binding proteins.

#### Distribution of GABA<sub>B</sub>R1 or GABA<sub>B</sub>R2 in cDNA Libraries

[0410] Three cDNA libraries were screened by PCR with primers directed to transmembrane regions of either GABA<sub>B</sub>R1 or GABA<sub>B</sub>R2. In a human hippocampal cDNA

library both polypeptides were found in greater than 90% of the pools and in a rat hypothalamic cDNA library, again both polypeptides were found in greater than 90% of the pools. In addition, within each of these two libraries, the respective frequency of GABA<sub>B</sub>R1 and GABA<sub>B</sub>R2 seems to be the same. However, in a rat spinal cord cDNA library, GABA<sub>B</sub>R1 was found in 62.5% of the pools while GABA<sub>B</sub>R2 was found in only 17.5% of the pools. Thus, while both polypeptide subtype appear to be present at high frequency in all three of the libraries, in the spinal cord library GABA<sub>B</sub>R2 occurs at 3.6-fold lower frequency. These data point to the existence of an additional GABA<sub>B</sub>-like peptide(s).

#### Results of Localization Controls

[0411] The specificity of the hybridization of the GABA<sub>B</sub>R2 probe was verified by performing in situ hybridization on transiently transfected HEK293 cells as described in Methods. The results indicate that hybridization to each of the individual GABA<sub>B</sub> polypeptides was specific only to the HEK293 cells transfected with each respective cDNA. In addition, in situ hybridization on rat brain sections was performed using two hybridization probes targeted to different segments of the GABA<sub>B</sub>R2 mRNA. In each case the pattern and intensity of labeling was identical in all regions of the rat CNS. Nonspecific hybridization signal was determined using the sense probes and was indistinguishable from background.

#### Localization of GABA<sub>B</sub>R2 mRNA in Rat CNS

[0412] The anatomical distribution of GABA<sub>B</sub>R2 mRNA in the rat brain was determined by in situ hybridization. By light microscopy the silver grains were determined to be distributed over neuronal profiles. The results suggest that the mRNA for GABA<sub>B</sub>R2 is widely distributed throughout the rat CNS in addition to several sensory ganglia (FIGS. 19H-I). However, expression levels in the brain were not uniform with several regions exhibiting higher levels of expression such as the medial habenula, CA3 region of the hippocampus, piriform cortex, and cerebellar Purkinje cells (FIGS. 19A-F). Moderate expression levels were observed in the ventral pallidum, septum, thalamus, CA1 region of the hippocampus, and geniculate nuclei (FIGS. 19C, D, E). Lower expression of GABA<sub>B</sub>R2 mRNA was seen in the hypothalamus, mesencephalon, and several brainstem nuclei (FIGS. 19D, F). GABAergic neurons and terminals are likewise widely distributed in the CNS (Mugnaini, E., et al., 1985). and the distribution of the GABA<sub>B</sub>R2 mRNA correlates well with the distribution of GABAergic neurons. One exception is the substantia nigra which contains high densities of GABAergic neurons, yet very low expression of GABA<sub>B</sub>R2 mRNA. Additionally, the anatomical distribution of GABA<sub>B</sub>R2 mRNA is in concordance with previous reports of the distribution of GABA<sub>B</sub> binding sites obtained using [<sup>3</sup>H]baclofen (Gehlert, D. R., et al., 1985), and [<sup>3</sup>H] GABA (Bowery, N. J., et al., 1987). Furthermore, there was a high degree of similarity in the distribution and intensity of GABA<sub>B</sub>R2 hybridization signal relative to those previously reported for GABA<sub>B</sub>R1 (Bischoff, S., et al., 1997) (FIGS. 11, 12). Notable exceptions were the hypothalamus and caudate-putamen, where the expression of GABA<sub>B</sub>R2 message appeared lower than that of GABA<sub>B</sub>R1.

### Colocalization of GABA<sub>B</sub>R2 and GABA<sub>B</sub>R1b mRNAs in the Rat CNS

[0413] The results of the in situ hybridization studies using digoxigenin-labeled probe conjugated to alkaline phosphatase and the chromagen NBT/BCIP for the GABA<sub>B</sub>R2 mRNA and an [<sup>35</sup>S]dATP-labeled probe for the GABA<sub>B</sub>R1b mRNA indicated that coexpression of the GABA<sub>B</sub>R2 mRNA and GABA<sub>B</sub>R1b mRNA did occur in vivo in neurons. In particular, colocalization was observed in cells of the medial habenula, hippocampus, and the cerebellar Purkinje cells. Likewise, there was evidence from the autoradiograms for potential overlapping distribution of the three known GABA<sub>B</sub>mRNAs in the olfactory bulb, throughout the entire neocortex, several hypothalamic nuclei, numerous thalamic nuclei and brain stem nuclei. However, the Purkinje cells of the cerebellum contained message for only GABA<sub>B</sub>R2 and GABA<sub>B</sub>R1b and not the GABA<sub>B</sub>R1a. Additionally, all three subtypes appear to be distributed throughout the gray matter of the spinal cord in all levels of the spinal cord.

[0414] The overlapping expression patterns of GABA<sub>B</sub>R1 and GABA<sub>B</sub>R2 transcripts in the brain suggested the possibility the polypeptides may be co-expressed in individual neurons and that both might be required for functional activity.

### Oocyte Expression

[0415] Postsynaptic inhibition of neurons by GABA<sub>B</sub> receptor activation is caused by the opening of inwardly rectifying K<sup>+</sup> channels (GIRK) (North, R. A., 1989; Andrade, R. et al., 1986; Gähwiler, B. H., et al., 1985; Luscher, C., et al., 1997). Oocytes expressing the combination of GABA<sub>B</sub>R1b and GABA<sub>B</sub>R2 mRNAs together with GIRKs elicited large currents in response to 30  $\mu$ M GABA (Table 1a and 1b). (Subsequent to the compilation of the data in Table 1a, experiments were done to make Table 1b.) GABA and baclofen evoked sustained currents of similar magnitude (FIG. 13B). In contrast, oocytes expressing transcripts encoding either GABA<sub>B</sub>R1a, GABA<sub>B</sub>R1b, or GABA<sub>B</sub>R2 alone consistently failed to generate GIRK currents in response to high concentrations of GABA (1 mM), baclofen (1 mM) or 3-APMPA (100  $\mu$ M). Others have reported similar results with GABA<sub>B</sub>R1 (Kaupmann, K. et al., 1997a; Kaupmann, K., et al., 1997b).

TABLE 1a

Magnitude of GIRK currents stimulated by GABA in oocytes and HEK-293 cells expressing GIRK1 and GIRK4 and various combinations of rat GABA <sub>B</sub> R1 and rat GABA <sub>B</sub> R2.						
Oocytes			HEK-293			
mean (nA)	S.E.M.	(n)	mean (pA)	S.E.M.	(n*)	
GABA <sub>B</sub> R1a	0	0 (35)	—	—	—	
GABA <sub>B</sub> R1b	0	0 (15)	5	3	(3/26)	
GABA <sub>B</sub> R2	0	0 (19)	5	5	(1/6)	
GABA <sub>B</sub> R1b+	1396	269 (7)	658	323	(9/10)	
GABA <sub>B</sub> R2						
GABA <sub>B</sub> R1b+	7	7 (2)	—	—	—	
GABA <sub>B</sub> R2+						
PTX						

\*number of cells responding/total number studied

[0416]

TABLE 1b

Magnitude of GIRK currents stimulated by GABA in oocytes and HEK-293 cells expressing GIRK1 and GIRK4 and various combinations of rat GABA <sub>B</sub> R1 and rat GABA <sub>B</sub> R2.					
Oocytes			HEK-293		
mean (nA)	S.E.M.	(n)	mean (pA)	S.E.M.	(n*)
GABA <sub>B</sub> R1a	0	0 (35)	—	—	—
GABA <sub>B</sub> R1b	0	0 (23)	5	3	(5/26)
GABA <sub>B</sub> R2	0.230	.13 (30)	.87	.87	(1/23)
GABA <sub>B</sub> R1b+	832	65 (65)	470	71	(70/81)
GABA <sub>B</sub> R2					
GABA <sub>B</sub> R1b+	16	9 (3)	—	—	—
GABA <sub>B</sub> R2+					
PTX					

\*number of cells responding/total number studied

[0417] Currents stimulated by GABA in oocytes injected with both GABA<sub>B</sub>R1b and GABA<sub>B</sub>R2 mRNAs were completely blocked by the selective antagonist CGP55845 (1  $\mu$ M) in a reversible fashion (data not shown). The potency of GABA and baclofen for eliciting GIRK currents was measured by performing steady-state cumulative concentration response assays on individual oocytes (FIG. 6A). Like K<sup>+</sup> responses elicited by stimulation of native GABA<sub>B</sub> receptors (Lacy et al. 1988; Misgeld et al. 1995), responses in oocytes did not desensitize and could be faithfully reproduced by multiple agonist applications on single oocytes. Stimulation of inward current was concentration dependent for both GABA and baclofen. The EC<sub>50</sub>s, 1.76  $\mu$ M for GABA and 3.99  $\mu$ M for baclofen (FIG. 6B, FIG. 7), agreed closely with those reported in the literature for native receptors (Lacy et al. 1988; Misgeld et al. 1995). Concentration-effect curves for GABA were shifted to the right, in an apparently competitive manner, by well characterized GABA<sub>B</sub>-selective antagonists (FIG. 15B). Based on additional experiments, the EC<sub>50</sub>'s are 1.32  $\mu$ M for GABA and 3.31  $\mu$ M for baclofen. The results to date are summarized in Table 2. Antagonist affinity estimates (FIG. 15B, Table 2) were similar to values reported in previous electrophysiological studies using brain tissue (Bon, C., et al., 1996; Seabrook, G. R., et al., 1990), as well as to those obtained by measuring displacement of radioligand from cells expressing GABA<sub>B</sub>R1 alone (Kaupmann, K., et al., 1997a) (Table 2).

TABLE 2

Agonist and antagonist pharmacology in cells expressing GABA <sub>B</sub> R1, GABA <sub>B</sub> R2, or both.						
Protein	Measurement Agonist			Antagonist		
	GABA	Bacl ofen	3-APMPA	PhacI ofen	CGP5 4626	CGP55 845
GABA <sub>B</sub> pEC <sub>50</sub> <sup>1</sup>	5.88 ±	5.48	7.29 ±	3.80	7.48 ±	8.60 ±
R1 + pK <sub>a</sub> <sup>2</sup>	0.01	±	0.02	±	0.05	0.09
GA		0.05		0.03 <sup>4</sup>		
BA <sub>B</sub> R2						
GABA <sub>B</sub> pK <sub>i</sub> <sup>3</sup>	4.6	4.3	5.2	>3.0	8.95	8.7
R1						

<sup>1</sup>n = 6–8 oocytes except for GABA; n = 20 oocytes.

<sup>2</sup>Measured using GABA as agonist; n = 4–6 oocytes.

<sup>3</sup>Displacement of [<sup>3</sup>H]-CGP54626 from COS-7 cells expressing

GABA<sub>B</sub>R1; n = 3.4

<sup>4</sup>IC<sub>50</sub> using EC<sub>50</sub> concentration of GABA.

[0418] Evidence that GABA-induced currents were mediated by GIRK channels included: 1) dependency on elevated external  $K^+$ , 2) strong inward rectification of the current-voltage ( $I/v$ ) relation, 3) reversal potential ( $-23.3$  mV) close to the predicted equilibrium potential for  $K^+$  ( $-23$  mV), and 4) sensitivity to block by  $100 \mu M$   $Ba^{++}$  (FIG. 8).

[0419] Three oocytes were injected with pertussis toxin (2 ng/oocyte) 6 h before voltage clamping. GABA-stimulated currents were abolished in these oocytes (Table 1a and 1b), suggesting that receptor activation of GIRKs was mediated by G-proteins  $G_i$  or  $G_o$ . Analogous results have been obtained by others expressing D2 dopamine receptors with GIRKs in oocytes (Werner et al. 1996).

#### GABA Responses in Co-transfected HEK-293 Cells.

[0420] To verify that both gene products,  $GABA_B R1b$  and  $GABA_B R2$ , are also required for expression of functional  $GABA_B$  receptors in mammalian cells, voltage clamp recordings were obtained from HEK-293 cells transiently transfected with various combinations of each gene along with GIRKs. Cells transfected with a combination of  $GABA_B R1b$  (BO58) and  $GABA_B R2$  (BO55) plus GIRKs consistently produced large  $K^+$  currents in response to  $100 \mu M$  GABA (9 of 10 cells tested, Table 1a and 70 of 81 cells tested, Table 1b). Large amplitude currents were also observed when  $GABA_B R2$  was paired with the  $GABA_B R1a$  splice variant ( $1046 \pm 247$  pA;  $n=9$ ). In contrast, cells transfected with only one of the  $GABA_B$  genes plus GIRKs responded either not at all or only very weakly to GABA (Table 1a and 1b). Small agonist-evoked currents ( $10$ - $50$  pA) were observed in 5 of 26 cells expressing  $GABA_B R1$ ; similar weak currents were evoked in 1 of 23 cells expressing  $GABA_B R2$ .

[0421] GABA-elicited currents in doubly transfected cells were completely blocked by  $100 \mu M$   $Ba^{++}$  or the competitive antagonist CGP55845 at  $1 \mu M$  (FIG. 9). The  $EC_{50}$  for GABA stimulation of GIRKs in HEK-293 cells was determined using similar methods as for oocytes. The  $EC_{50}$ ,  $3.42 \mu M$ , was comparable to that measured in oocytes ( $1.76 \mu M$ ; further experiments gave  $1.32 \mu M$ ). Thus, whether in *Xenopus* oocytes or HEK-293 cells, the behavior of the  $GABA_B$  receptor is the same. Co-expression of both  $GABA_B R1b$  and  $GABA_B R2$  is required to observe activation of the receptor by GABA.

[0422] To determine if co-expressed  $GABA_B R1/R2$  could mediate a cellular response in the absence of exogenously supplied GIRKs, we transiently co-transfected CHO cells with  $GABA_B R1$  and  $GABA_B R2$  and measured agonist-evoked extracellular acidification using a microphysiometer. Baclofen stimulated a 9-fold increase in acidification rate (FIG. 16) which was blocked by  $100$  nM CGP55845 and by pretreatment with PTX (not shown). This response was absent in cells expressing either protein alone. Since GIRK activity is undetectable in wild-type CHO cells (Krapivinsky, G., et al., 1995b) we conclude that GIRK expression is not a prerequisite for signal generation by  $GABA_B R1/R2$ .

#### $GABA_B R1/GABA_B R2$ Signaling Through Chimeric G-proteins

[0423] Chimeric G-proteins have been used to "switch" the coupling pathway of a GPCR from one that normally inhibits adenylyl cyclase to one that activates phospholipase C (Conklin et al., 1993). With the aim of developing an assay based on  $Ca^{++}$  or some other signal amenable to high throughput screening, we employed a  $G\alpha_{q/13}$  chimera to obtain  $Ca^{++}$ -induced  $Cl^-$  responses in oocytes. Oocytes were injected with  $GABA_B R1$  and  $GABA_B R2$  mRNAs as previously described. 2-3 days later oocytes were injected again with  $50$  pg of  $G\alpha_{q/13}$  mRNA and recorded under voltage clamp conditions. In response to GABA ( $0.1$ - $1$  mM) 88% of these oocytes produced rapidly desensitizing inward currents ( $454 \pm 92$  nA;  $n=14$ ) typical of those stimulated by receptors that normally couple to  $G\alpha_q$ . In contrast, oocytes injected with only the  $GABA_B R1/GABA_B R2$  combination ( $n>100$ ), or  $GABA_B R1$  plus  $G\alpha_{q/13}$  ( $n=4$ ) failed to produce currents.

[0424]  $GABA_B$  agonists also resulted in concentration-dependent stimulation of phosphoinositide production in COS-7 cells transfected transiently with  $GABA_B R1$ ,  $GABA_B R2$ , and the chimeric G-protein  $G\alpha_{q/13}$ . The concentration of agonist evoking 50% of its maximum response ( $EC_{50}$ ) and fold stimulation over basal were: GABA ( $EC_{50}=1.8 \mu M$ ; 2.4 fold); baclofen ( $1.7 \mu M$ ; 1.8 fold); 3-aminopropylmethylphosphonic acid ( $EC_{50}=0.11 \mu M$ ; 2.2 fold). These results indicate that G-protein chimeras, in particular  $G\alpha_{q/13}$  and  $G\alpha_{q/13}$ , are useful for directing  $GABA_B$  receptor stimulation to a phosphoinositide- or  $Ca^{++}$ -based assay.

[0425] A comparison of the pharmacological properties of  $GABA_B R1$  and  $GABA_B R2$  using radioligand binding revealed that membranes from HEK293 or COS-7 cells expressing  $GABA_B R1$ , but not those expressing  $GABA_B R2$ , were labeled by the high affinity antagonist [ $^3H$ ]-CGP54626<sup>21</sup> (Table 2), indicating that the polypeptides are pharmacologically distinct. Neither was labeled by the agonists [ $^3H$ ]-GABA or [ $^3H$ ]-baclofen. Furthermore, with the available ligands (GABA, baclofen, APMPA, phaclofen, CGP54626, CGP-55845 and SCH-50911) the binding profile of membranes from cells co-transfected with  $GABA_B R1/R2$  was not different from those transfected with  $GABA_B R1$  alone. The absence of detectable high affinity agonist binding to  $GABA_B R1/R2$ , as well as to  $GABA_B R1b$ , constitutes a notable distinction from the  $GABA_B$  binding profile in the CNS and may reflect the absence of an essential, as yet undefined G-protein or accessory protein.

[0426] The molecular mechanism by which protein co-expression confers functional activity is unknown. We noted that varying the ratios of  $GABA_B R1/R2$  cDNAs from 1/100 to 100/1 in HEK293 cells resulted in a symmetrical fall off in response amplitude (FIG. 14B). This suggests that a 1:1 protein stoichiometry may be critical, and caused us to postulate that the polypeptides are forming a heteromeric association. Biochemical evidence supports the idea that certain GPCRs can exist as homodimers (Hebert, T. E., et al.,

1996; Cvejic, S., et al., 1997; Ciruela, F., et al., 1995; Avissar, S., et al., 1983; Romano, C., et al., 1996), but the functional significance of this has been largely unexplored (Hebert, T. E., et al., 1996; Wreggett, K. A., et al., 1995). The possibility of a physical association was investigated using epitope-tagged versions of GABA<sub>B</sub>R1 (RGS6xH tag) and GABA<sub>B</sub>R2 (HA tag). C-terminal modification did not appear to alter the function of either polypeptide; maximal current amplitudes (FIG. 14B) and EC<sub>50</sub> values for GABA (4.97  $\mu$ M, n=5) were unchanged compared to HEK293 cells expressing the wild-type GABA<sub>B</sub>R1/R2 receptor combination (3.42  $\mu$ M, n=5). The subcellular distribution of epitope-tagged proteins was examined in transfected cells by fluorescence microscopy. When expressed individually, GABA<sub>B</sub>R1<sup>RGS6xH</sup> and GABA<sub>B</sub>R2<sup>HA</sup> were localized throughout the plasma membrane. Optical sectioning of antibody-labeled cells by confocal microscopy confirmed the membrane localization pattern, with less labeling in the cytoplasm and none in the nucleus. In co-transfected cells there was a striking overlap in the distribution of the two epitope tags (FIG. 17A-17C). Both proteins were prominently expressed on the plasma membrane. Furthermore, co-localization occurred within the cytoplasm, suggesting that GABA<sub>B</sub>R1 and GABA<sub>B</sub>R2 assemble in the endoplasmic reticulum. In contrast, the cellular distribution of an unrelated GPCR, NPY Y5, differed considerably from that of GABA<sub>B</sub>R2 (FIG. 17D), suggesting specificity in the association of GABA<sub>B</sub>R2 with GABA<sub>B</sub>R1.

[0427] Western blots of whole cell extracts from cells expressing GABA<sub>B</sub>R1<sup>RGS6xH</sup>, GABA<sub>B</sub>R2<sup>HA</sup> or both, exhibited bands close to the predicted molecular weights of the two proteins (92 kD for GABA<sub>B</sub>R1, 97 kD for GABA<sub>B</sub>R2) and additional bands corresponding to the predicted molecular weights of receptor dimers (FIG. 18A,B). To determine if GABA<sub>B</sub>R1 and GABA<sub>B</sub>R2 co-associate in a heteromeric complex, we immunoprecipitated solubilized material from cells expressing both polypeptides. GABA<sub>B</sub>R2<sup>HA</sup> was detected in material immunoprecipitated using either anti-His or anti-HA antibodies (FIG. 18). To determine if GABA<sub>B</sub>R1b and GABA<sub>B</sub>R2 co-associate in a heteromeric complex, we performed immunoprecipitations using membrane fractions enriched in plasma membrane as determined by the presence of Na<sup>+</sup>/K<sup>+</sup> ATPase (FIG. 20A). In co-transfected cells only, GABA<sub>B</sub>R2<sup>HA</sup> was detected in material immunoprecipitated using antibodies specific for the GABA<sub>B</sub>R1<sup>RGS6xH</sup> protein (FIG. 20B). This result confirms that both GABA<sub>B</sub>R1 and GABA<sub>B</sub>R2 are correctly targeted to the plasma membrane of HEK293 cells, and that the two proteins exist in a heteromeric complex, perhaps as heterodimers, on the membrane surface.

#### Responses Measured Using a Microphysiometer

[0428] The results described so far using rat clones of GABA<sub>B</sub>R1 and GABA<sub>B</sub>R2 indicate that heterodimer formation is critical for the activation of GABA<sub>B</sub> receptors by agonists. Co-expression experiments with human polypeptides suggest that the same phenomenon occurs for human homologues of GABA<sub>B</sub>R1 and GABA<sub>B</sub>R2. For example, using the microphysiometer we found that only when both rat GABA<sub>B</sub>R1 and rat GABA<sub>B</sub>R2 were co-expressed was there a dose-dependent acidification response to baclofen. In other experiments when human GABA<sub>B</sub>R1 was substituted for rat GABA<sub>B</sub>R1, and co-expressed with rat GABA<sub>B</sub>R2, a similar concentration-dependent acidification response was

observed (FIG. 24). The calculated EC<sub>50</sub> values for baclofen were indistinguishable between rat GABA<sub>B</sub>R1/rat GABA<sub>B</sub>R2 (1.09  $\mu$ M) and human GABA<sub>B</sub>R1/rat GABA<sub>B</sub>R2 (1.08  $\mu$ M). These data strongly support the notion that like the rat receptor, the human GABA<sub>B</sub> receptor is formed by subunits composed of GABA<sub>B</sub>R1 and GABA<sub>B</sub>R2.

#### GABA<sub>B</sub> Mediated Calcium Responses Measured Using FLIPR

[0429] Confluent monolayers of COS-7 cells in 150 cm<sup>2</sup> flasks were transfected simultaneously with three individual plasmids containing (1) the human GABA<sub>B</sub>R1 (5 ng), (2) the human GABA<sub>B</sub>R2 (5 ng), and (3) the chimeric G-protein G $\alpha_{q13}$  (10 ng), using the DEAE/Dextran method (Lopata, et al., 1984). The cells were grown under controlled conditions for 24 h, at which time they were re-seeded into poly-D-lysine coated 96 well plates (Biocoat, VWR #62406-036) at a density of 50000 cells/well, and grown for another 24 h.

[0430] On the day of the assay, the medium was aspirated and 100  $\mu$ l of loading buffer (Hank's buffer containing 20 mM HEPES, 1% FBS, 2.5 mM probenecid, and 4  $\mu$ M Fluo-3  $\mu$ M; F1241 Molecular Probes, Eugene, Oreg.) was added to each well. The cells were then incubated for 1 h at 37° C. in an atmosphere of 5% CO<sub>2</sub>. The cells were then washed 3x with assay buffer (Hank's buffer containing 20 mM HEPES and 2.5 mM probenecid) at room temperature. The cells were left in a final volume of 150 assay buffer. To begin the assay, test compounds were added from drug plates in 50  $\mu$ l volumes via the FLIPR instrument (Molecular Devices, Sunnyvale, Calif.) in a volume of 50  $\mu$ l. Receptor-mediated increases in intracellular Ca<sup>2+</sup> concentration were recorded as fluorescent units. After subtracting baseline fluorescence, responses above background were plotted versus agonist concentration to give final concentration effect curves. Concentration-effect curves were analyzed by non-linear regression analysis to derive agonist potencies.

[0431] The GABA<sub>B</sub> agonist 3-APMPA evoked concentration dependent increases in intracellular Ca<sup>2+</sup> concentration in COS-7 cells transfected with GABA<sub>B</sub>R1, GABA<sub>B</sub>R2, and G $\alpha_{q13}$ . The potency derived for 3-APMPA in this assay was 6.85 (pEC<sub>50</sub>).

#### Experimental Discussion

[0432] A gene has been cloned that shows 38% overall identity at the amino acid level with the recently cloned GABA<sub>B</sub>R1 polypeptide. Important predicted features of the new gene product include 7 transmembrane spanning regions, and a large extracellular N-terminal domain. Like the GABA<sub>B</sub>R1 gene product, GABA<sub>B</sub>R2 by itself does not promote the activation of cellular effectors such as GIRKs. When co-expressed together, however, the two permit a GABA<sub>B</sub> receptor phenotype that is quite similar to that found in the brain. The functional attributes of this reconstituted receptor include: 1) robust stimulation of a physiological effector (GIRKs), 2) EC<sub>50</sub>s for GABA and baclofen in the same range as for GABA<sub>B</sub> receptors previously studied in the CNS, 3) antagonism by the high affinity selective antagonist CGP55845, and 4) inhibition of receptor function by pertussis toxin. These attributes are not observed when either GABA<sub>B</sub>R1 or GABA<sub>B</sub>R2 is expressed alone.

[0433] Our data indicate that GABA<sub>B</sub>R1 and GABA<sub>B</sub>R2 associate as subunits to produce a single pharmacologically

and functionally defined receptor. Consistent with this view, double labeling in situ hybridization experiments provided evidence that GABA<sub>B</sub>R1 and GABA<sub>B</sub>R2 mRNAs are co-expressed in individual neurons and populations of neurons in several regions of the nervous system including hippocampal pyramidal cells (FIG. 21), cerebellar Purkinje cells (FIG. 12A,B) and sensory neurons in mesencephalic trigeminal nucleus (FIG. 21) and dorsal root ganglia. This co-localization pattern of GABA<sub>B</sub>R1 and R2 transcripts predicts that GABA<sub>B</sub> receptors on these cells are comprised of GABA<sub>B</sub>R1/R2 heteromers. Other as yet unidentified GABA<sub>B</sub> receptor homologues may associate elsewhere to produce novel subtypes. For example, the low level of expression of GABA<sub>B</sub>R2 mRNA relative to GABA<sub>B</sub>R1 in caudate putamen and hypothalamus (FIGS. 11A,B) raises the possibility that other GABA<sub>B</sub> receptor homologues may associate with GABA<sub>B</sub>R1 to produce novel subtypes in these regions. Conclusive evidence that functional GABA<sub>B</sub> receptors exist in vivo as multimers will await immunofluorescence studies with specific antibodies.

[0434] The recent cloning of a family of accessory proteins that modify the binding and functional properties of a calcitonin-receptor-like receptor (McLarchie, et al., 1998) demonstrates that some 7-TM spanning proteins require additional unrelated proteins to reconstitute native GPCR activity. GABA<sub>B</sub>R1 and GABA<sub>B</sub>R2 are the first examples of 7-TM proteins for which activity is dependent on an interaction with another member within the same family of proteins. There will be considerable interest in whether other GPCRs are formed by heteromeric complexes of related 7-TM proteins. Many members of the superfamily of GPCRs, such as D<sub>3</sub>, 5-HT<sub>2</sub>, and olfactory receptors, do not function well in heterologous expression systems and may require related partners to generate native receptor function (Nimchinsky, et al., 1997). The growing list of receptors that have been reported to exist as homodimers (Ciruela, F., et al., 1995; Cvejic, S., et al., 1997; Hebert, T. E., et al., 1996; Romano, C., et al., 1996; Maggio, R., et al., 1996) points to the likelihood that both homomeric and heteromeric assemblies are more widespread among GPCRs than previously thought.

[0435] There are several possible explanations for why two genes are required for full function of the GABA<sub>B</sub> receptor. One possible explanation is that the two gene products function together as a heterodimer having high affinity agonist and antagonist binding sites. Currently, there is no precedent for heterodimerization of GPCRs. There is evidence that certain GPCRs, for example the mGluR5 receptor, can form homodimers via cysteine disulfide bridges in the N-terminal domain (Romano et al., 1996).

[0436] Significantly, synthetic peptides that inhibit homodimerization of beta2-adrenergic receptors also reduce agonist stimulation of adenyl cyclase activity (Hebert et al., 1996). Useful parallels may be drawn from other classes of receptors where heterodimeric structures are well-known. For example, the NMDA (glutamate) receptor is comprised of two principal subunits, neither of which alone permits all of the native features of the receptor (see Wisden and Seeburg, 1993). GABA<sub>B</sub> receptors may be comprised simi-

larly of two (or more) peptide subunits, such as GABA<sub>B</sub>R1 and GABA<sub>B</sub>R2, that form a quaternary structure having appropriate binding sites for agonist and G-protein.

[0437] A role for GABA<sub>B</sub>R2 in modulating sensory information is suggested by in situ hybridization histochemistry which revealed the expression of GABA<sub>B</sub>R2 mRNA in relay nuclei of several sensory pathways. In the olfactory and visual pathways GABA<sub>B</sub>R2 appears to be in a position to modulate excitatory glutamatergic projections from the olfactory bulb and retina. GABA<sub>B</sub>R2 mRNA was observed in the target regions of projection fibers from the main olfactory bulb, including the olfactory tubercle, piriform and entorhinal cortices and from the retina, for instance the superior colliculus (FIGS. 19A,B; Table 3).

[0438] The ability to modulate nociceptive information might be indicated not only by the presence of GABA<sub>B</sub>R2 transcripts in somatic sensory neurons of the trigeminal and dorsal root ganglia (FIGS. 19H-I) but also by being present in the target regions of nociceptive primary afferent fibers, including the superficial layers of the spinal trigeminal nucleus and dorsal horn of the spinal cord (FIGS. 19F-G). Again, in each of these loci GABA<sub>B</sub>R2 has been shown to be in a position to potentially modulate the influence of excitatory glutamatergic nociceptive primary afferents. In both ganglia, microscopic examination indicated that the hybridization signal did not appear to be restricted to any one size cell and was distributed evenly over small, medium and large ganglion cells. Thus, GABA<sub>B</sub>R2 may be able to influence various sensory modalities. Expression levels appeared to be higher in the ganglion cells of the dorsal root with light to moderate expression in the trigeminal ganglia.

[0439] GABA<sub>B</sub>R2 mRNA was likewise observed to be expressed in the vestibular nuclei which are target regions of inhibitory GABAergic Purkinje cells and also in the Purkinje cells themselves, suggesting that GABA<sub>B</sub>R2 may be important in the mediation of planned movements (FIG. 19F).

[0440] Moderate expression of GABA<sub>B</sub>R2 transcripts throughout the telencephalon indicate a potential modulatory role in the processing of somatosensory and limbic system (entorhinal cortex) information, in addition to modulating visual (parietal cortex) and auditory stimuli (temporal cortex) as well as cognition. Furthermore, modulation of patterns of integrated behaviors, such as defense, ingestion, aggression, reproduction and learning could also be attributed to this receptor owing to its expression in the amygdala (Table 3). The high levels of expression in the thalamus suggest a possible regulatory role in the transmission of somatosensory (nociceptive) information to the cortex and the exchange of information between the forebrain and midbrain limbic system (habenula). The presence of GABA<sub>B</sub>R2 mRNA in the hypothalamus indicates a likely modulatory role in food intake, reproduction, the expression of emotion and possibly neuroendocrine regulation (FIG. 19D). A role in the mediation of memory acquisition and learning may be suggested by the presence of the GABA<sub>B</sub>R2 transcript throughout all regions of the hippocampus and the entorhinal cortex (FIG. 19D).

TABLE 3

Distribution of rGABA<sub>B</sub>R2, rGABA<sub>B</sub>R1a, and GABA<sub>B</sub>1b mRNA in the rat CNS. The strength of the hybridization signal for each of the respective mRNAs obtained in various regions of the rat brain was graded as weak (+), moderate (++) , heavy (+++) or intense (++++ ) and is relative to the individual polypeptides.

Region	GABA <sub>B</sub> R2	GABA <sub>B</sub> R1a*	GABA <sub>B</sub> R1b*	Potential Application
Olfactory bulb				Modulation of olfactory sensation
internal granule layer	+	++	++	
glomerular layer	+	++	++	
external plexiform layer	—	—	—	
mitral cell layer	—	+	++	
anterior olfactory n	++	++	++	
olfactory tubercle	+	++	+++	
Islands of Calleja	—	++	+++	
Telencephalon				Sensory integration
taenia	++	++	++	
tecta				
frontal cortex	++	++	++	
orbital cortex	++	++	++	
agranular insular cortex	+++	++	++	
cingulate cortex	++	++	+	
retrosplenial cortex	++	++	+	
parietal cortex	++	++	++	Processing of visual stimuli
occipital cortex	++	++	++	
temporal cortex	++	++	++	Processing of auditory stimuli
perirhinal cortex	++	++		
entorhinal cortex	++	++	++	Processing of visceral information
dorsal endopiriform n	++	++	++	
piriform cortex	+++	+++	+++	Integration/transmission of incoming olfactory information
<u>Basal Ganglia</u>				
accumbens n	+	++	++	Modulation of dopaminergic function
caudateputamen	+	+	++	Sensory/motor integration
globus pallidus	+	—	+	
<u>Septum</u>				
medial septum	++	++	+	Cognitive enhancement via cholinergic system



TABLE 3-continued

Distribution of rGABA <sub>B</sub> R2, rGABA <sub>B</sub> R1a, and GABA <sub>B</sub> 1b mRNA in the rat CNS. The strength of the hybridization signal for each of the respective mRNAs obtained in various regions of the rat brain was graded as weak (+), moderate (++) , heavy (+++) or intense (++++ ) and is relative to the individual polypeptides.				
Region	GABA <sub>B</sub> R2	GABA <sub>B</sub> R1a*	GABA <sub>B</sub> R1b*	Potential Application
lateral septum	++	+	++	Modulation of integration of stimuli associated with adaptation
septohippocampal n	+	+	+++	
diagonal band n	++	++	++	
ventral pallidum	++	+	+	
Amygdala				Anxiolytic (activation - reduction in panic attacks) appetite, depression
basolateral n	++	+	+	
medial amygdaloid n	+	+	+	Olfactory amygdala
basomedial n	+	+	+	
central n	+++	—	+	
anterior cortical n	+	+	+	
posteromedial cortical n	++	+	+	
bed n stria terminalis	++	+	++	
zona incerta	+	+	+	
Hippocampus				Memory consolidation and retention
CA1 , Ammon's horn	++	+++	+++	
CA2 , Ammon's horn	++++	+++	+++	
CA3 , Ammon's horn	++++	+++	+++	Facilitation of LTP
subiculum	+	+++	+++	
parasubiculum	++	++	++	
presubiculum	++	++	++	
dentate gyrus	++++	+++	++	
polymorph dentate gyrus	+++	+++	++	
Hypothalamus				
suprachiasmatic n	+	++	ND	
median preoptic area	+	+	++	Regulation of gonadotropin secretion and reproductive behaviors
paraventricular n	+	++	++	Appetite/obesity
arcuate n	++	++	++	
anterior hypoth., post lateral hypoth.	+	+	++	
ventromedial n	+	++	+++	
periventricular n	+	+	+	
supraoptic n	+	++	+	Synthesis of OXY and AVP
supramammillary n	++	++	++	Modulation of hypothalamic projections to cortex

TABLE 3-continued

Distribution of rGABA <sub>B</sub> R2, rGABA <sub>B</sub> R1a, and GABA <sub>B</sub> 1b mRNA in the rat CNS. The strength of the hybridization signal for each of the respective mRNAs obtained in various regions of the rat brain was graded as weak (+), moderate (++) , heavy (+++) or intense (++++) and is relative to the individual polypeptides.				
Region	GABA <sub>B</sub> R2	GABA <sub>B</sub> R1a*	GABA <sub>B</sub> R1b*	Potential Application
premamillary n	+	+	+	Analgesia/Modulation of sensory information
medial	+	++	+	
mamillary n				
Thalamus				
paraventricular n	++	+	++	Modulation of motor and behavioral responses to pain
centromedial n	++	+	++	Modulation of motor and behavioral responses to pain
paracentral n.	++	+	++	Modulation of motor and behavioral responses to pain
parafascicular n	++	+	++	
anterodorsal n	+++	+	++	Modulation of eye movement
laterodorsal n	+++	+	++	Modulation of thalamic input to ventral hippocampus and entorhinal ctx
lateral	++	+	++	
posterior n				
reuniens n	+++	+	++	
rhomboid n	+++	+	++	Anxiety/sleep disorders/analgesia in chronic pain
medial habenula	++++	+	++++	
lateral habenula	+	+	+++	Alertness/sedation
ventrolateral n	+++	+	++	
ventromedial n	+++	++	++	
ventral	+++	+	++	
posterolateral n				Modulation of visual perception
reticular n	++	+	+	
lateral	++	+	++	
geniculate n				
medial	++	+	++	Modulation of auditory system
geniculate				
subthalamic n	++	++	++	
Mesencephalon				
superior colliculus	+	+	+	Modulation of vision
inferior colliculus	+	+	+	
central gray	+	+	+	Analgesia
dorsal raphe	+	++	+	
deep	+	+	+	
mesencephalic n				
phalic n				
oculomotor n	+			
pontine n	+++		++	
retrotrubral field	+			

TABLE 3-continued

Distribution of rGABA <sub>B</sub> R2, rGABA <sub>B</sub> R1a, and GABA <sub>B</sub> 1b mRNA in the rat CNS. The strength of the hybridization signal for each of the respective mRNAs obtained in various regions of the rat brain was graded as weak (+), moderate (++), heavy (+++) or intense (++++), and is relative to the individual polypeptides.				
Region	GABA <sub>B</sub> R2	GABA <sub>B</sub> R1a*	GABA <sub>B</sub> R1b*	Potential Application
ventral tegmental area	+	++	++	Modulation of the integration of motor behavior and adaptive responses Motor control
substantia nigra, reticular	+	+	+	
substantia nigra, compact	++	++	++	
interpeduncular n	++	ND	ND	Analgesia Analgesia
Myelencephalon raphe magnus	++		++	
raphe pallidus	+	++	ND	
principal trigeminal	+	+		
spinal trigeminal n	+	+	+	
pontine reticular n	++	+	++	
parvocellular reticular n	+	++	++	
locus coeruleus	++	++	++	Modulation of NA transmission
parabrachial n	+	++	+	Modulation of visceral sensory information Maintenance of balance and equilibrium
vestibular n	+	++	+	Inhibition and disinhibition of brainstem
gigantocellular reticular n	+	++	++	Position and movement of the eyes/ Modulation of arterial pressure and heart rate
prepositus hypoglossal n	+	+++	++	
ventral cochlear n	++	+	ND	Hypertension
n solitary tract	++		ND	
A5 Noradrenergic cells	+	ND	+	
facial n(7)	+	++		Motor coordination, Autism
Cerebellum				
granule cell layer	+	+	+	
Purkinje cells	++	—	++	
Spinal cord dorsal horn	+	++	+	Analgesia
ventral horn	+	++	+	
trigeminal ganglion	++	+++	+	Nociception
dorsal root ganglion	++++	+++	ND	Nociception

ND = not determined

\*Bischoff S et al.

## List of Abbreviations

- [0441] 7 facial n
- [0442] ac anterior commissure
- [0443] Acb accumbens n
- [0444] ACo anterior cortical amygdaloid n
- [0445] AI agranular insular cortex
- [0446] AON anterior olfactory n
- [0447] APir amygdalopiriform transition area
- [0448] APT anterior pretectal n
- [0449] Arc arcuate hypothalamic n
- [0450] BLA basolateral amygdaloid n
- [0451] CA1-3 Fields of Ammon's horn
- [0452] cc corpus callosum
- [0453] Cg cingulate cortex
- [0454] CeA central amygdaloid n
- [0455] CPU caudate-putamen
- [0456] DG dentate gyrus
- [0457] DLG dorsal lateral geniculate n
- [0458] DpMc deep mesencephalic n
- [0459] Ent entorhinal cortex
- [0460] Gi gigantocellular reticular n
- [0461] Gr granule cell layer, cerebellum
- [0462] GrO granule layer olf. bulb
- [0463] FrA frontal association cortex
- [0464] GP globus pallidus
- [0465] HDB horizontal diagonal band
- [0466] LA lateral amygdaloid n
- [0467] LH lateral hypothalamus
- [0468] LO lateral orbital cortex
- [0469] LV lateral ventricle
- [0470] M1 primary motor cortex
- [0471] MeAD medial amygdaloid n, anterodorsal
- [0472] MG medial geniculate
- [0473] MHb medial habenular n
- [0474] MPO medial preoptic n
- [0475] PC Purkinje cell layer of the cerebellum
- [0476] PF parafascicular n
- [0477] Pir piriform cortex
- [0478] PMCO posteromedial cortical amygdaloid n
- [0479] Pr prepositus n
- [0480] PVA paraventricular thalamic n
- [0481] RS retrosplenial cortex
- [0482] S subiculum

- [0483] SFi septofimbrial n
- [0484] SI substantia innominata
- [0485] SNc substantia nigra, compact
- [0486] STh subthalamic n
- [0487] Sp5 spinal trigeminal n
- [0488] TT tenia tecta
- [0489] Ve vestibular n
- [0490] VTA ventral tegmental area

Potential Therapeutic Application for GABA<sub>B</sub> Agonists and Antagonists

## Agonists

## Antinociception

[0491] A potential GABA<sub>B</sub> agonist application may in antinociception. The inhibitory effects of GABA and GABA<sub>B</sub> agonists are thought to be predominantly a presynaptic mechanism on excitation-induced impulses in high threshold Ad and C fibers on primary afferents. This effect can be blocked by GABA<sub>B</sub> antagonists (Hao, J.-H., et al., 1994). Baclofen's spinal cord analgesic effects have been well documented in the rat, though it has not been as effective in human. However, baclofen has been successful in the treatment of trigeminal neuralgia in human.

[0492] The localization of the GABA<sub>B</sub>R2 mRNA in the superficial layers of the spinal cord dorsal horn, the termination site for primary afferents, as well as their cells of origin in the dorsal root and trigeminal ganglia position the GABA<sub>B</sub>R1/R2 receptor appropriately for mediating the agonist effects.

## Drug Addiction

[0493] It has been suggested that GABA agonists may have some potential in the treatment of cocaine addiction. A role for the action of psychostimulants in the mesoaccumbens dopamine system is well established. The ventral pallidum receives a GABAergic projection from the nucleus accumbens and both regions contain GABA<sub>B</sub>R2 transcripts. GABA receptors were shown to have an inhibitory effect on dopamine release in the ventral pallidum. Phaclofen acting at these receptors resulted in increased dopamine release and baclofen was shown to attenuate the reinforcing effects of cocaine. (Roberts, D. C. S., et al., 1996; Morgan, A. E. et al.)

## Micturition

[0494] There is a potential application for GABA<sub>B</sub> agonists in the treatment of bladder dysfunction. Baclofen has been used in the treatment of detrusor hyperreflexia through inhibition of contractile responses. In addition to a peripheral site of action for GABA<sub>B</sub> agonists, there is also the possibility for a central site. The pontine micturition center in the brainstem is involved in mediating the spinal reflex pathway, via Onuf's nucleus in the sacral spinal cord. Support for possible application of GABA<sub>B</sub> agonists in the treatment of bladder dysfunction may be augmented by presence of GABA<sub>B</sub>R2 mRNA in the various nuclei involved in the control of the lower urinary tract function.

## Antagonists

## Memory Enhancement—Alzheimer's Disease

[0495] GABA<sub>B</sub> antagonists may have a potential application in the treatment of Alzheimer's Disease. The blockade of GABA<sub>B</sub> receptors might lead to signal amplification and improvement in cognitive functions resulting from an increased excitability of cortical neurons via amplification of the acetylcholine signal. Additionally, memory may be enhanced by GABA<sub>B</sub> antagonists which have been shown to suppress late IPSPs, thus facilitating long-term potentiation in the hippocampus (see Table 3).

[0496] To support this idea, CGP36742, a GABA<sub>B</sub> antagonist, has been shown to improve learning performance in aged rats as well as the performance of rhesus monkeys in conditioned spatial color task. (Mondadori, C. et al., 1993). The significance of the GABA<sub>B</sub>R1/R2 receptor in cognitive functioning might be indicated by the presence of GABA<sub>B</sub>R2 mRNA in the cerebral cortex and its codistribution in the ventral forebrain with cortically projecting cholinergic neurons as well as its localization in the pyramidal cells in all regions of Ammon's horn and dentate gyrus in the hippocampus.

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Phe Phe Arg Thr Val Pro Ser Asp Asn Ala Val Asn Pro Ala Ile Leu
130          135          140
Lys Leu Leu Lys His Tyr Gln Trp Lys Arg Val Gly Thr Leu Thr Gln
145          150          155          160
Asp Val Gln Arg Phe Ser Glu Val Arg Asn Asp Leu Thr Gly Val Leu
165          170          175
Tyr Gly Glu Asp Ile Glu Ile Ser Asp Thr Glu Ser Phe Ser Asn Asp
180          185          190
Pro Cys Thr Ser Val Lys Lys Leu Lys Gly Asn Asp Val Arg Ile Ile
195          200          205
Leu Gly Gln Phe Asp Gln Asn Met Ala Ala Lys Val Phe Cys Cys Ala
210          215          220
Tyr Glu Glu Asn Met Tyr Gly Ser Lys Tyr Gln Trp Ile Ile Pro Gly
225          230          235          240
Trp Tyr Glu Pro Ser Trp Trp Glu Gln Val His Thr Glu Ala Asn Ser
245          250          255
Ser Arg Cys Leu Arg Lys Asn Leu Leu Ala Ala Met Glu Gly Tyr Ile
260          265          270
Gly Val Asp Phe Glu Pro Leu Ser Ser Lys Gln Ile Lys Thr Ile Ser
275          280          285

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Gly	Lys	Thr	Pro	Gln	Gln	Tyr	Glu	Arg	Glu	Tyr	Asn	Asn	Lys	Arg	Ser
290						295					300				
Gly	Val	Gly	Pro	Ser	Lys	Phe	His	Gly	Tyr	Ala	Tyr	Asp	Gly	Ile	Trp
305					310					315					320
Val	Ile	Ala	Lys	Thr	Leu	Gln	Arg	Ala	Met	Glu	Thr	Leu	His	Ala	Ser
				325					330					335	
Ser	Arg	His	Gln	Arg	Ile	Gln	Asp	Phe	Asn	Tyr	Thr	Asp	His	Thr	Leu
			340					345					350		
Gly	Arg	Ile	Ile	Leu	Asn	Ala	Met	Asn	Glu	Thr	Asn	Phe	Phe	Gly	Val
	355						360					365			
Thr	Gly	Gln	Val	Val	Phe	Arg	Asn	Gly	Glu	Arg	Met	Gly	Thr	Ile	Lys
	370					375					380				
Phe	Thr	Gln	Phe	Gln	Asp	Ser	Arg	Glu	Val	Lys	Val	Gly	Glu	Tyr	Asn
385					390					395					400
Ala	Val	Ala	Asp	Thr	Leu	Glu	Ile	Ile	Asn	Asp	Thr	Ile	Arg	Phe	Gln
				405					410					415	
Gly	Ser	Glu	Pro	Pro	Lys	Asp	Lys	Thr	Ile	Ile	Leu	Glu	Gln	Leu	Arg
			420					425					430		
Lys	Ile	Ser	Leu	Pro	Leu	Tyr	Ser	Ile	Leu	Ser	Ala	Leu	Thr	Ile	Leu
	435						440					445			
Gly	Met	Ile	Met	Ala	Ser	Ala	Phe	Leu	Phe	Phe	Asn	Ile	Lys	Asn	Arg
	450					455					460				
Asn	Gln	Lys	Leu	Ile	Lys	Met	Ser	Ser	Pro	Tyr	Met	Asn	Asn	Leu	Ile
465					470					475					480
Ile	Leu	Gly	Gly	Met	Leu	Ser	Tyr	Ala	Ser	Ile	Phe	Leu	Phe	Gly	Leu
				485					490					495	
Asp	Gly	Ser	Phe	Val	Ser	Glu	Lys	Thr	Phe	Glu	Thr	Leu	Cys	Thr	Val
			500					505					510		
Arg	Thr	Trp	Ile	Leu	Thr	Val	Gly	Tyr	Thr	Thr	Ala	Phe	Gly	Ala	Met
	515						520					525			
Phe	Ala	Lys	Thr	Trp	Arg	Val	His	Ala	Ile	Phe	Lys	Asn	Val	Lys	Met
	530					535					540				
Lys	Lys	Lys	Ile	Ile	Lys	Asp	Gln	Lys	Leu	Leu	Val	Ile	Val	Gly	Gly
545					550					555					560
Met	Leu	Leu	Ile	Asp	Leu	Cys	Ile	Leu	Ile	Cys	Trp	Gln	Ala	Val	Asp
			565					570					575		
Pro	Leu	Arg	Arg	Thr	Val	Glu	Lys	Tyr	Ser	Met	Glu	Pro	Asp	Pro	Ala
			580					585					590		
Gly	Arg	Asp	Ile	Ser	Ile	Arg	Pro	Leu	Leu	Glu	His	Cys	Glu	Asn	Thr
	595						600					605			
His	Met	Thr	Ile	Trp	Leu	Gly	Ile	Val	Tyr	Ala	Tyr	Lys	Gly	Leu	Leu
	610					615					620				
Met	Leu	Phe	Gly	Cys	Phe	Leu	Ala	Trp	Glu	Thr	Arg	Asn	Val	Ser	Ile
625				630					635						640
Pro	Ala	Leu	Asn	Asp	Ser	Lys	Tyr	Ile	Gly	Met	Ser	Val	Tyr	Asn	Val
			645						650					655	
Gly	Ile	Met	Cys	Ile	Ile	Gly	Ala	Ala	Val	Ser	Phe	Leu	Thr	Arg	Asp
		660					665					670			
Gln	Pro	Asn	Val	Gln	Phe	Cys	Ile	Val	Ala	Leu	Val	Ile	Ile	Phe	Cys
	675						680					685			
Ser	Thr	Ile	Thr	Leu	Cys	Leu	Val	Phe	Val	Pro	Lys	Leu	Ile	Thr	Leu

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690	695	700
Arg Thr Asn Pro Asp Ala Ala Thr Gln Asn Arg Arg Phe Gln Phe Thr 705 710 715 720		
Gln Asn Gln Lys Lys Glu Asp Ser Lys Thr Ser Thr Ser Val Thr Ser 725 730 735		
Val Asn Gln Ala Ser Thr Ser Arg Leu Glu Gly Leu Gln Ser Glu Asn 740 745 750		
His Arg Leu Arg Met Lys Ile Thr Glu Leu Asp Lys Asp Leu Glu Glu 755 760 765		
Val Thr Met Gln Leu Gln Asp Thr Pro Glu Lys Thr Thr Tyr Ile Lys 770 775 780		
Gln Asn His Tyr Gln Glu Leu Asn Asp Ile Leu Asn Leu Gly Asn Phe 785 790 795 800		
Thr Glu Ser Thr Asp Gly Gly Lys Ala Ile Leu Lys Asn His Leu Asp 805 810 815		
Gln Asn Pro Gln Leu Gln Trp Asn Thr Thr Glu Pro Ser Arg Thr Cys 820 825 830		
Lys Asp Pro Ile Glu Asp Ile Asn Ser Pro Glu His Ile Gln Arg Arg 835 840 845		
Leu Ser Leu Gln Leu Pro Ile Leu His His Ala Tyr Leu Pro Ser Ile 850 855 860		
Gly Gly Val Asp Ala Ser Cys Val Ser Pro Cys Val Ser Pro Thr Ala 865 870 875 880		
Ser Pro Arg His Arg His Val Pro Pro Ser Phe Arg Val Met Val Ser 885 890 895		

Gly Leu

<210> SEQ ID NO 3  
 <211> LENGTH: 2823  
 <212> TYPE: DNA  
 <213> ORGANISM: Rattus Sp.

&lt;400&gt; SEQUENCE: 3

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tggacgcggg gcgccccccg gccgcccgc agcagcccgc cgctctccat catgggcctc	180
atgccgctca ccaaggaggt ggccaagggc agcatcgggc gcggcggtgt ccccgccgtg	240
gagctagcca tcgagcagat ccgcaacgag tcaactcctgc gccctactt cctggacctg	300
cgactctatg acaccgagtg tgacaatgca aagggactga aagccttcta tgacgcaata	360
aagtatgggc cgaaccattt gatggtgtt ggaggcgtct gtccgtctgt cacatctatt	420
atcgcgaggt cctccaagg ctggaatctg gtgcagcttt ccttcgcgc caccacgcct	480
gttcttgccg ataagaagaa gtaccgtat ttcttcggga cggtgccgtc agacaacgcg	540
gtgaaccccg ccatcctgaa gctcctgaag cacttccgct ggcggcggtg gggcacactc	600
acgcaggacg tgcagcgctt ctccgagtg aggaatgacc tgactggggt tctgtatggg	660
gaagatatgt agatctcaga cacagagagt ttctccaatg atccctgcac cagcgtcaaa	720
aagctcaagg ggaatgacgt gcggatcatc cttggccagt ttgaccagaa tatggcagca	780
aaagtcttct gttgtgcctt cgaggagagc atgtttggca gcaagtacca gtggatcatc	840

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cgggagtggt acgagcctgc gtggtgggag caggtgcatg tggaggccaa ttcctcacgc	900
tgcctgcgca gaagcctcct ggctgccatg gaaggttaca tcggagtga ctttgagccc	960
ctgagctcca aacaaatcaa gaccatctca gggaagactc cacagcagta tgaaagagag	1020
tacaacagca aacgttcagg cgtggggccc agcaagttcc atgggtacgc ctacgatggg	1080
atctgggtca tcgccaaagac cctacagagg gccatggaga cactgcatgc cagtagcagg	1140
caccagcgga tccaggactt caactacaca gaccacacgc tgggcanaat catcctcaat	1200
gccatgaacg agaccaactt ctctggggtc acgggtcaag ttgtgttccg gaacggggag	1260
agaatgggaa ccattaaatt tactcaattt caagacagca gagaggtgaa ggtcggcgaa	1320
tacaacgcgg tggctgacac actggagatc atcaatgaca ccataaggtt ccagggttcc	1380
gagccacca aggacaagac catcattctg gagcagcttc ggaagatctc gcttccactg	1440
tatagcatcc tgtccgtctc caccatctc gccatgatca tggccagcgc ctctctcttc	1500
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ctcatcatcc tgggaggaat gctgtctat gcatccatct tctcttttg cctcgatggg	1620
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cgcccatctg tggaaactg cgaaaacacc cacatgacca tctggcttg cattgtctac	1980
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tgcacgtggt ccctgggtcat catcttctgc agcaccatca ctctctgcct ggtgtttgtg	2220
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cccagctccc agtggaaacac gacagagccc tcaagaacat gcaaagacc catagaagac	2640
atcaactccc cggagcacat ccagcgccgg ctgtcgtctc agctcccat ccttcaccac	2700
gcctacctcc catccatcgg aggcgtggat gccagctgcg tcagccctg tgcagccct	2760
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tag	2823

<210> SEQ ID NO 4  
 <211> LENGTH: 929  
 <212> TYPE: PRT  
 <213> ORGANISM: Rattus Sp.  
 <400> SEQUENCE: 4

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Met	Ala	Ser	Pro	Pro	Ser	Ser	Gly	Gln	Pro	Arg	Pro	Pro	Pro	Pro	Pro	1	5	10	15
Pro	Pro	Pro	Ala	Arg	Leu	Leu	Leu	Pro	Leu	Leu	Leu	Ser	Leu	Leu	Leu	20	25	30	
Trp	Leu	Ala	Pro	Gly	Ala	Trp	Gly	Trp	Thr	Arg	Gly	Ala	Pro	Arg	Pro	35	40	45	
Pro	Pro	Ser	Ser	Pro	Pro	Leu	Ser	Ile	Met	Gly	Leu	Met	Pro	Leu	Thr	50	55	60	
Lys	Glu	Val	Ala	Lys	Gly	Ser	Ile	Gly	Arg	Gly	Val	Leu	Pro	Ala	Val	65	70	75	80
Glu	Leu	Ala	Ile	Glu	Gln	Ile	Arg	Asn	Glu	Ser	Leu	Leu	Arg	Pro	Tyr	85	90	95	
Phe	Leu	Asp	Leu	Arg	Leu	Tyr	Asp	Thr	Glu	Cys	Asp	Asn	Ala	Lys	Gly	100	105	110	
Leu	Lys	Ala	Phe	Tyr	Asp	Ala	Ile	Lys	Tyr	Gly	Pro	Asn	His	Leu	Met	115	120	125	
Val	Phe	Gly	Gly	Val	Cys	Pro	Ser	Val	Thr	Ser	Ile	Ile	Ala	Glu	Ser	130	135	140	
Leu	Gln	Gly	Trp	Asn	Leu	Val	Gln	Leu	Ser	Phe	Ala	Ala	Thr	Thr	Pro	145	150	155	160
Val	Leu	Ala	Asp	Lys	Lys	Lys	Tyr	Pro	Tyr	Phe	Phe	Arg	Thr	Val	Pro	165	170	175	
Ser	Asp	Asn	Ala	Val	Asn	Pro	Ala	Ile	Leu	Lys	Leu	Leu	Lys	His	Phe	180	185	190	
Arg	Trp	Arg	Arg	Val	Gly	Thr	Leu	Thr	Gln	Asp	Val	Gln	Arg	Phe	Ser	195	200	205	
Glu	Val	Arg	Asn	Asp	Leu	Thr	Gly	Val	Leu	Tyr	Gly	Glu	Asp	Ile	Glu	210	215	220	
Ile	Ser	Asp	Thr	Glu	Ser	Phe	Ser	Asn	Asp	Pro	Cys	Thr	Ser	Val	Lys	225	230	235	240
Lys	Leu	Lys	Gly	Asn	Asp	Val	Arg	Ile	Ile	Leu	Gly	Gln	Phe	Asp	Gln	245	250	255	
Asn	Met	Ala	Ala	Lys	Val	Phe	Cys	Cys	Ala	Phe	Glu	Glu	Ser	Met	Phe	260	265	270	
Gly	Ser	Lys	Tyr	Gln	Trp	Ile	Ile	Pro	Gly	Trp	Tyr	Glu	Pro	Ala	Trp	275	280	285	
Trp	Glu	Gln	Val	His	Val	Glu	Ala	Asn	Ser	Ser	Arg	Cys	Leu	Arg	Arg	290	295	300	
Ser	Leu	Leu	Ala	Ala	Met	Glu	Gly	Tyr	Ile	Gly	Val	Asp	Phe	Glu	Pro	305	310	315	320
Leu	Ser	Ser	Lys	Gln	Ile	Lys	Thr	Ile	Ser	Gly	Lys	Thr	Pro	Gln	Gln	325	330	335	
Tyr	Glu	Arg	Glu	Tyr	Asn	Ser	Lys	Arg	Ser	Gly	Val	Gly	Pro	Ser	Lys	340	345	350	
Phe	His	Gly	Tyr	Ala	Tyr	Asp	Gly	Ile	Trp	Val	Ile	Ala	Lys	Thr	Leu	355	360	365	
Gln	Arg	Ala	Met	Glu	Thr	Leu	His	Ala	Ser	Ser	Arg	His	Gln	Arg	Ile	370	375	380	
Lys	Ile	Ile	Leu	Asn	Ala	Met	Asn	Glu	Thr	Asn	Phe	Phe	Gly	Val	Thr	385	390	395	400
Gly	Gln	Val	Val	Phe	Arg	Asn	Gly	Glu	Arg	Met	Gly	Thr	Ile	Lys	Phe				

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405					410					415					
Thr	Gln	Phe	Gln	Asp	Ser	Arg	Glu	Val	Lys	Val	Gly	Glu	Tyr	Asn	Ala
			420					425					430		
Val	Ala	Asp	Thr	Leu	Glu	Ile	Ile	Asn	Asp	Thr	Ile	Arg	Phe	Gln	Gly
			435					440					445		
Ser	Glu	Pro	Pro	Lys	Asp	Lys	Thr	Ile	Ile	Leu	Glu	Gln	Leu	Arg	Lys
			450					455					460		
Ile	Ser	Leu	Pro	Leu	Tyr	Ser	Ile	Leu	Ser	Ala	Leu	Thr	Ile	Leu	Gly
			465					470					475		480
Met	Ile	Met	Ala	Ser	Ala	Phe	Leu	Phe	Phe	Asn	Ile	Lys	Asn	Arg	Asn
			485					490					495		
Gln	Lys	Leu	Ile	Lys	Met	Ser	Ser	Pro	Tyr	Met	Asn	Asn	Leu	Ile	Ile
			500					505					510		
Leu	Gly	Gly	Met	Leu	Ser	Tyr	Ala	Ser	Ile	Phe	Leu	Phe	Gly	Leu	Asp
			515					520					525		
Gly	Ser	Phe	Val	Ser	Glu	Lys	Thr	Phe	Glu	Thr	Leu	Cys	Thr	Val	Arg
			530					535					540		
Thr	Trp	Ile	Leu	Thr	Val	Gly	Tyr	Thr	Thr	Ala	Phe	Gly	Ala	Met	Phe
			545					550					555		560
Ala	Lys	Thr	Trp	Arg	Val	His	Ala	Ile	Phe	Lys	Asn	Val	Lys	Met	Lys
			565					570					575		
Lys	Lys	Ile	Ile	Lys	Asp	Gln	Lys	Leu	Leu	Val	Ile	Val	Gly	Gly	Met
			580					585					590		
Leu	Leu	Ile	Asp	Leu	Cys	Ile	Leu	Ile	Cys	Trp	Gln	Ala	Val	Asp	Pro
			595					600					605		
Leu	Arg	Arg	Thr	Val	Glu	Arg	Tyr	Ser	Met	Glu	Pro	Asp	Pro	Ala	Gly
			610					615					620		
Arg	Asp	Ile	Ser	Ile	Arg	Pro	Leu	Leu	Glu	His	Cys	Glu	Asn	Thr	His
			625					630					635		640
Met	Thr	Ile	Trp	Leu	Gly	Ile	Val	Tyr	Ala	Tyr	Lys	Gly	Leu	Leu	Met
			645					650					655		
Leu	Phe	Gly	Cys	Phe	Leu	Ala	Trp	Glu	Thr	Arg	Asn	Val	Ser	Ile	Pro
			660					665					670		
Ala	Leu	Asn	Asp	Ser	Lys	Tyr	Ile	Gly	Met	Ser	Val	Tyr	Asn	Val	Gly
			675					680					685		
Ile	Met	Cys	Ile	Ile	Gly	Ala	Ala	Val	Ser	Phe	Leu	Thr	Arg	Asp	Gln
			690					695					700		
Pro	Asn	Val	Gln	Phe	Cys	Ile	Val	Ala	Leu	Val	Ile	Ile	Phe	Cys	Ser
			705					710					715		720
Thr	Ile	Thr	Leu	Cys	Leu	Val	Phe	Val	Pro	Lys	Leu	Ile	Thr	Leu	Arg
			725					730					735		
Thr	Asn	Pro	Asp	Ala	Ala	Thr	Gln	Asn	Arg	Arg	Phe	Gln	Phe	Thr	Gln
			740					745					750		
Asn	Gln	Lys	Lys	Glu	Asp	Ser	Lys	Thr	Ser	Thr	Ser	Val	Thr	Ser	Val
			755					760					765		
Asn	Gln	Ala	Ser	Thr	Ser	Arg	Leu	Glu	Gly	Leu	Gln	Ser	Glu	Asn	His
			770					775					780		
Arg	Leu	Arg	Met	Lys	Ile	Thr	Glu	Leu	Asp	Lys	Asp	Leu	Glu	Glu	Val
			785					790					795		800
Thr	Met	Gln	Leu	Gln	Asp	Thr	Pro	Glu	Lys	Thr	Thr	Tyr	Ile	Lys	Gln
			805					810					815		

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Asn His Tyr Gln Glu Leu Asn Asp Ile Leu Ser Leu Gly Asn Phe Thr  
                     820                    825                    830  
 Glu Ser Thr Asp Gly Gly Lys Ala Ile Leu Lys Asn His Leu Asp Gln  
                     835                    840                    845  
 Asn Pro Gln Leu Gln Trp Asn Thr Thr Glu Pro Ser Arg Thr Cys Lys  
                     850                    855                    860  
 Asp Pro Ile Glu Asp Ile Asn Ser Pro Glu His Ile Gln Arg Arg Leu  
                     865                    870                    875                    880  
 Ser Leu Gln Leu Pro Ile Leu His His Ala Tyr Leu Pro Ser Ile Gly  
                     885                    890                    895  
 Gly Val Asp Ala Ser Cys Val Ser Pro Cys Val Ser Pro Thr Ala Ser  
                     900                    905                    910  
 Pro Arg His Arg His Val Pro Pro Ser Phe Arg Val Met Val Ser Gly  
                     915                    920                    925  
 Leu

<210> SEQ ID NO 5  
 <211> LENGTH: 45  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence:  
                     Oligonucleotide Probe

<400> SEQUENCE: 5

agggatgctt tcctatgctt ccatatttct ctttggcctt gatgg

45

<210> SEQ ID NO 6  
 <211> LENGTH: 45  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence:  
                     Oligonucleotide Probe

<400> SEQUENCE: 6

caatgtgcag ttctgcatcg tggctctgggt catcatcttc tgcag

45

<210> SEQ ID NO 7  
 <211> LENGTH: 24  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 7

cttctaggcc tgtacggaag tggt

24

<210> SEQ ID NO 8  
 <211> LENGTH: 26  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 8

gttgtggttt gtccaaactc atcaat

26

<210> SEQ ID NO 9

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<211> LENGTH: 24  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
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<400> SEQUENCE: 9

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<210> SEQ ID NO 10  
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<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 10

tgcgttgctg catctgggtt tgttct 26

<210> SEQ ID NO 11  
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<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 11

atctccctac ctctctacag catcct 26

<210> SEQ ID NO 12  
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<213> ORGANISM: Artificial Sequence  
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<400> SEQUENCE: 12

caggtcctga cggtgcaaag tgtttc 26

<210> SEQ ID NO 13  
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<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 13

tgacgcaaga cgttcagagg ttctct 26

<210> SEQ ID NO 14  
<211> LENGTH: 26  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 14

tgtagccttc catggcagca agcaga 26

<210> SEQ ID NO 15  
<211> LENGTH: 26  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:



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<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 15

agagaacctc tgaacgtctt gcgtca

26

<210> SEQ ID NO 16

<211> LENGTH: 26

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 16

ggctctgttg tgttcactg tagctg

26

<210> SEQ ID NO 17

<211> LENGTH: 26

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 17

tcâtggccgct caccaaggag gtggcc

26

<210> SEQ ID NO 18

<211> LENGTH: 26

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 18

ggccacctcc ttggtgagcg gcatga

26

<210> SEQ ID NO 19

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 19

tgagtgaaca gagtccagag ccgt

24

<210> SEQ ID NO 20

<211> LENGTH: 26

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 20

atggatggga ggtaggcgtg gtggag

26

<210> SEQ ID NO 21

<211> LENGTH: 26

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 21

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ctctctgccc tcaccatcct cgggat

26

<210> SEQ ID NO 22  
<211> LENGTH: 26  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

&lt;400&gt; SEQUENCE: 22

gactccggct cgaataccag gcagag

26

<210> SEQ ID NO 23  
<211> LENGTH: 27  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

&lt;400&gt; SEQUENCE: 23

ccatgtttgc aaagacctgg aggggtcc

27

<210> SEQ ID NO 24  
<211> LENGTH: 27  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

&lt;400&gt; SEQUENCE: 24

ggtcacgcgt caggaaagag acagcag

27

<210> SEQ ID NO 25  
<211> LENGTH: 25  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

&lt;400&gt; SEQUENCE: 25

aagcttctag agatccctcg acctc

25

<210> SEQ ID NO 26  
<211> LENGTH: 25  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

&lt;400&gt; SEQUENCE: 26

aggcgagaa ctggtaggta tggaa

25

<210> SEQ ID NO 27  
<211> LENGTH: 25  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

&lt;400&gt; SEQUENCE: 27

cttctaggcc tgtacggaag tgta

25

&lt;210&gt; SEQ ID NO 28

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<211> LENGTH: 27  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer  
  
<400> SEQUENCE: 28  
gttgtgtgttt gtccaaactc atcaatg 27  
  
<210> SEQ ID NO 29  
<211> LENGTH: 27  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer  
  
<400> SEQUENCE: 29  
ctgtgtgtctc ttctctgacg cgtgacc 27  
  
<210> SEQ ID NO 30  
<211> LENGTH: 59  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer  
  
<400> SEQUENCE: 30  
ccaagcttct aatacgactc actatagggg agaccatggg cccgggggga ccctgtacc 59  
  
<210> SEQ ID NO 31  
<211> LENGTH: 63  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer  
  
<400> SEQUENCE: 31  
tttttttttt tttttttttt tttttttttt tttttcactt gtaaagcaaa tgtactcgac 60  
tcc 63  
  
<210> SEQ ID NO 32  
<211> LENGTH: 37  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer  
  
<400> SEQUENCE: 32  
cgcggtatcca ttatgtctgc actccgaagg aaatttg 37  
  
<210> SEQ ID NO 33  
<211> LENGTH: 38  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer  
  
<400> SEQUENCE: 33  
cgcgaattct tatgtgaagc gatcagagtt catttttc 38  
  
<210> SEQ ID NO 34  
<211> LENGTH: 34  
<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 34

gcgggatccg ctatggctgg tgattctagg aatg 34

<210> SEQ ID NO 35  
<211> LENGTH: 29  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 35

ccggaattcc cctcacaccg agcccttg 29

<210> SEQ ID NO 36  
<211> LENGTH: 44  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence:  
Oligonucleotide Probe

<400> SEQUENCE: 36

gcaataaagt atgggctgaa ccatttgatg gtgtttggag gcgt 44

<210> SEQ ID NO 37  
<211> LENGTH: 44  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence:  
Oligonucleotide Probe

<400> SEQUENCE: 37

acgcctccaa acaccatcaa atggttcagc ccatacttta ttgc 44

<210> SEQ ID NO 38  
<211> LENGTH: 40  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence:  
Oligonucleotide Probe

<400> SEQUENCE: 38

tttgagcccc tgagctccaa acaaatcaag accatctcag 40

<210> SEQ ID NO 39  
<211> LENGTH: 40  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence:  
Oligonucleotide Probe

<400> SEQUENCE: 39

ctgagatggt cttgatttgt ttggagctca ggggctcaaa 40

<210> SEQ ID NO 40  
<211> LENGTH: 43  
<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence:  
Oligonucleotide Probe

<400> SEQUENCE: 40

aaggccatca acttcttgcc tgtggactat gagatcgaat atg 43

<210> SEQ ID NO 41  
<211> LENGTH: 43  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence:  
Oligonucleotide Probe

<400> SEQUENCE: 41

catattcgat ctcatagtcc acaggcagga agttgatggc ctt 43

<210> SEQ ID NO 42  
<211> LENGTH: 38  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence:  
Oligonucleotide Probe

<400> SEQUENCE: 42

tggccgctgc ctcttctgct ggtgatggcg gctgggggt 38

<210> SEQ ID NO 43  
<211> LENGTH: 38  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence:  
Oligonucleotide Probe

<400> SEQUENCE: 43

accccagccg ccatcaccag cagaagaggc agcggcca 38

<210> SEQ ID NO 44  
<211> LENGTH: 45  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence:  
Oligonucleotide Probe

<400> SEQUENCE: 44

ccttggtttt ggccttgaac aagacgtctg gaggaggtgg tcgtt 45

<210> SEQ ID NO 45  
<211> LENGTH: 45  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence:  
Oligonucleotide Probe

<400> SEQUENCE: 45

aacgaccacc tcttcagac gtcttggtta aggccaaagc caagg 45

<210> SEQ ID NO 46

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&lt;211&gt; LENGTH: 2826

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Homo Sapiens

&lt;400&gt; SEQUENCE: 46

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atggcttccc cgcggagctc cgggcagccc ggccgcgcgc cgcgcgcgc accgcgcgcc 60
gcgcgcctgc tactgtact gctgctgcc ctgctgctgc ctctggcgcc cggggcctgg 120
ggctgggcgc ggggcgcccc ccggccgcgc ccagcagcc cgcgcgtctc catcatgggc 180
ctcatgccgc tcaccaagga ggtggccaag ggcagcatcg ggcgcggtgt gctccccgcc 240
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ctgcggctct atgacacgga gtgcgacaac gcaaaagggt tgaagcctt ctacgatgcg 360
ataaaatagc ggccgaacca cttgatgggt ttggaggcgc tctgtccatc cgtcacatcc 420
atcattgcag agtccctcca aggctggaat ctggtgcagc tttcttttgc tgcaaccacg 480
cctgttctag ccgataagaa aaaataccct tatttcttcc ggaccgtccc atcagacaat 540
gcggtgaatc cagccattct gaagttgctc aagcactacc agtggaaagc cgtgggcacg 600
ctgacgcgaag acgttcagag gttctctgag gtgcggaatg acctgactgg agttctgtat 660
ggcgaggaca ttgagatttc agacaccgag agcttctcca acgatccctg taccagtgtc 720
aaaaagctga aggggaatga tgtgcggatc atccttggcc agtttgacca gaatatggca 780
gcaaaagtgt tctgttggtc atacgaggag aacatgtatg gtagtaata tcagtggatc 840
attccgggct ggtacgagcc ttcttgggtg gagcaggtgc acacggaagc caactcatcc 900
cgctgcctcc ggaagaatct gcttgcctgc atggagggct acattggcgt ggatttcgag 960
cccctgagct ccaagcagat caagaccatc tcaggaaaga ctccacagca gtatgagaga 1020
gagtacaaca acaagcggtc aggcgtgggg ccagcaagt tccacgggta cgcctacgat 1080
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aatgccatga acgagaccaa cttcttcggg gtcacgggtc aagttgtatt ccggaatggg 1260
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gtggggggca tgctgctgat cgacctgtgt atcctgatct gctggcaggc tgtggacccc 1860
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tatgcctaca agggacttct catgtgttc ggtgtttct tagcttggga gaccgcgaac 2040
gtcagcatcc ccgcactcaa cgacagcaag tacatcggga tgagtgtcta caacgtgggg 2100

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atcatgtgca tcacggggc cgtgtctcc ttcctgacc gggaccagcc caatgtgcag 2160
ttctgcatcg tggctctggt catcatcttc tgcagcacca tcacctctg cctgggtatc 2220
gtgccgaagc tcacaccct gagaacaaac ccagatgcag caacgcagaa caggcgatc 2280
cagttcactc agaatcagaa gaaagaagat tctaaaacgt ccacctcggt caccagtgtg 2340
aaccaagcca gcacatcccg cctggagggc ctacagtcag aaaaccatcg cctgcgaatg 2400
aagatcacag agctggataa agacttgga gaggtcacca tgcagctgca ggacacacca 2460
gaaaagacca cctacattaa acagaaccac taccaagagc tcaatgacat cctcaacctg 2520
ggaaacttca ctgagagcac agatggagga aaggccattt taaaaaatca cctcgatcaa 2580
aatccccagc tacagtggaa cacaacagag cctctcga catgcaaaga tcctatagaa 2640
gatataaact ctccagaaca catccagcgt cggtgtccc tccagctccc catcctccac 2700
cagcctacc tccatccat cggaggcgtg gacgccagct gtgtcagccc ctgcgtcagc 2760
cccaccgcca gccccgcca cagacatgtg ccacctcct tccagtcac ggtctcgggc 2820
ctgtaa 2826

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&lt;210&gt; SEQ ID NO 47

&lt;211&gt; LENGTH: 941

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Homo Sapiens

&lt;400&gt; SEQUENCE: 47

```

Met Ala Ser Pro Arg Ser Ser Gly Gln Pro Gly Pro Pro Pro Pro Pro
 1             5             10             15
Pro Pro Pro Pro Ala Arg Leu Leu Leu Leu Leu Leu Leu Pro Leu Leu
 20             25             30
Leu Pro Leu Ala Pro Gly Ala Trp Gly Trp Ala Arg Gly Ala Pro Arg
 35             40             45
Pro Pro Pro Ser Ser Pro Pro Leu Ser Ile Met Gly Leu Met Pro Leu
 50             55             60
Thr Lys Glu Val Ala Lys Gly Ser Ile Gly Arg Gly Val Leu Pro Ala
 65             70             75             80
Val Glu Leu Ala Ile Glu Gln Ile Arg Asn Glu Ser Leu Leu Arg Pro
 85             90             95
Tyr Phe Leu Asp Leu Arg Leu Tyr Asp Thr Glu Cys Asp Asn Ala Lys
100            105            110
Gly Leu Lys Ala Phe Tyr Asp Ala Ile Lys Tyr Gly Pro Asn His Leu
115            120            125
Met Val Phe Gly Gly Val Cys Pro Ser Val Thr Ser Ile Ile Ala Glu
130            135            140
Ser Leu Gln Gly Trp Asn Leu Val Gln Leu Ser Phe Ala Ala Thr Thr
145            150            155            160
Pro Val Leu Ala Asp Lys Lys Lys Tyr Pro Tyr Phe Phe Arg Thr Val
165            170            175
Pro Ser Asp Asn Ala Val Asn Pro Ala Ile Leu Lys Leu Leu Lys His
180            185            190
Tyr Gln Trp Lys Arg Val Gly Thr Leu Thr Gln Asp Val Gln Arg Phe
195            200            205
Ser Glu Val Arg Asn Asp Leu Thr Gly Val Leu Tyr Gly Glu Asp Ile
210            215            220

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Glu	Ile	Ser	Asp	Thr	Glu	Ser	Phe	Ser	Asn	Asp	Pro	Cys	Thr	Ser	Val
225					230					235					240
Lys	Lys	Leu	Lys	Gly	Asn	Asp	Val	Arg	Ile	Ile	Leu	Gly	Gln	Phe	Asp
				245					250					255	
Gln	Asn	Met	Ala	Ala	Lys	Val	Phe	Cys	Cys	Ala	Tyr	Glu	Glu	Asn	Met
		260						265						270	
Tyr	Gly	Ser	Lys	Tyr	Gln	Trp	Ile	Ile	Pro	Gly	Trp	Tyr	Glu	Pro	Ser
		275					280					285			
Trp	Trp	Glu	Gln	Val	His	Thr	Glu	Ala	Asn	Ser	Ser	Arg	Cys	Leu	Arg
		290				295					300				
Lys	Asn	Leu	Leu	Ala	Ala	Met	Glu	Gly	Tyr	Ile	Gly	Val	Asp	Phe	Glu
305					310					315					320
Pro	Leu	Ser	Ser	Lys	Gln	Ile	Lys	Thr	Ile	Ser	Gly	Lys	Thr	Pro	Gln
				325					330					335	
Gln	Tyr	Glu	Arg	Glu	Tyr	Asn	Asn	Lys	Arg	Ser	Gly	Val	Gly	Pro	Ser
			340					345					350		
Lys	Phe	His	Gly	Tyr	Ala	Tyr	Asp	Gly	Ile	Trp	Val	Ile	Ala	Lys	Thr
		355					360					365			
Leu	Gln	Arg	Ala	Met	Glu	Thr	Leu	His	Ala	Ser	Ser	Arg	His	Gln	Arg
		370				375					380				
Ile	Gln	Asp	Phe	Asn	Tyr	Thr	Asp	His	Thr	Leu	Gly	Arg	Ile	Ile	Leu
385					390					395					400
Asn	Ala	Met	Asn	Glu	Thr	Asn	Phe	Phe	Gly	Val	Thr	Gly	Gln	Val	Val
				405					410					415	
Phe	Arg	Asn	Gly	Glu	Arg	Met	Gly	Thr	Ile	Lys	Phe	Thr	Gln	Phe	Gln
			420					425					430		
Asp	Ser	Arg	Glu	Val	Lys	Val	Gly	Glu	Tyr	Asn	Ala	Val	Ala	Asp	Thr
		435					440					445			
Leu	Glu	Ile	Ile	Asn	Asp	Thr	Ile	Arg	Phe	Gln	Gly	Ser	Glu	Pro	Pro
		450				455				460					
Lys	Asp	Lys	Thr	Ile	Ile	Leu	Glu	Gln	Leu	Arg	Lys	Ile	Ser	Leu	Pro
465					470					475					480
Leu	Tyr	Ser	Ile	Leu	Ser	Ala	Leu	Thr	Ile	Leu	Gly	Met	Ile	Met	Ala
				485					490					495	
Ser	Ala	Phe	Leu	Phe	Phe	Asn	Ile	Lys	Asn	Arg	Asn	Gln	Lys	Leu	Ile
			500					505					510		
Lys	Met	Ser	Ser	Pro	Tyr	Met	Asn	Asn	Leu	Ile	Ile	Leu	Gly	Gly	Met
			515				520					525			
Leu	Ser	Tyr	Ala	Ser	Ile	Phe	Leu	Phe	Gly	Leu	Asp	Gly	Ser	Phe	Val
			530			535					540				
Ser	Glu	Lys	Thr	Phe	Glu	Thr	Leu	Cys	Thr	Val	Arg	Thr	Trp	Ile	Leu
545					550					555					560
Thr	Val	Gly	Tyr	Thr	Thr	Ala	Phe	Gly	Ala	Met	Phe	Ala	Lys	Thr	Trp
			565					570						575	
Arg	Val	His	Ala	Ile	Phe	Lys	Asn	Val	Lys	Met	Lys	Lys	Lys	Ile	Ile
			580					585					590		
Lys	Asp	Gln	Lys	Leu	Leu	Val	Ile	Val	Gly	Gly	Met	Leu	Leu	Ile	Asp
			595				600					605			
Leu	Cys	Ile	Leu	Ile	Cys	Trp	Gln	Ala	Val	Asp	Pro	Leu	Arg	Arg	Thr
		610				615					620				
Val	Glu	Lys	Tyr	Ser	Met	Glu	Pro	Asp	Pro	Ala	Gly	Arg	Asp	Ile	Ser



625				630				635				640			
Ile	Arg	Pro	Leu	Leu	Glu	His	Cys	Glu	Asn	Thr	His	Met	Thr	Ile	Trp
645								650				655			
Leu	Gly	Ile	Val	Tyr	Ala	Tyr	Lys	Gly	Leu	Leu	Met	Leu	Phe	Gly	Cys
660								665				670			
Phe	Leu	Ala	Trp	Glu	Thr	Arg	Asn	Val	Ser	Ile	Pro	Ala	Leu	Asn	Asp
675								680				685			
Ser	Lys	Tyr	Ile	Gly	Met	Ser	Val	Tyr	Asn	Val	Gly	Ile	Met	Cys	Ile
690								695				700			
Ile	Gly	Ala	Ala	Val	Ser	Phe	Leu	Thr	Arg	Asp	Gln	Pro	Asn	Val	Gln
705								710				720			
Phe	Cys	Ile	Val	Ala	Leu	Val	Ile	Ile	Phe	Cys	Ser	Thr	Ile	Thr	Leu
725								730				735			
Cys	Leu	Val	Phe	Val	Pro	Lys	Leu	Ile	Thr	Leu	Arg	Thr	Asn	Pro	Asp
740								745				750			
Ala	Ala	Thr	Gln	Asn	Arg	Arg	Phe	Gln	Phe	Thr	Gln	Asn	Gln	Lys	Lys
755								760				765			
Glu	Asp	Ser	Lys	Thr	Ser	Thr	Ser	Val	Thr	Ser	Val	Asn	Gln	Ala	Ser
770								775				780			
Thr	Ser	Arg	Leu	Glu	Gly	Leu	Gln	Ser	Glu	Asn	His	Arg	Leu	Arg	Met
785								790				795			
Lys	Ile	Thr	Glu	Leu	Asp	Lys	Asp	Leu	Glu	Glu	Val	Thr	Met	Gln	Leu
805								810				815			
Gln	Asp	Thr	Pro	Glu	Lys	Thr	Thr	Tyr	Ile	Lys	Gln	Asn	His	Tyr	Gln
820								825				830			
Glu	Leu	Asn	Asp	Ile	Leu	Asn	Leu	Gly	Asn	Phe	Thr	Glu	Ser	Thr	Asp
835								840				845			
Gly	Gly	Lys	Ala	Ile	Leu	Lys	Asn	His	Leu	Asp	Gln	Asn	Pro	Gln	Leu
850								855				860			
Gln	Trp	Asn	Thr	Thr	Glu	Pro	Ser	Arg	Thr	Cys	Lys	Asp	Pro	Ile	Glu
865								870				875			
Asp	Ile	Asn	Ser	Pro	Glu	His	Ile	Gln	Arg	Arg	Leu	Ser	Leu	Gln	Leu
885								890				895			
Pro	Ile	Leu	His	His	Ala	Tyr	Leu	Pro	Ser	Ile	Gly	Gly	Val	Asp	Ala
900								905				910			
Ser	Cys	Val	Ser	Pro	Cys	Val	Ser	Pro	Thr	Ala	Ser	Pro	Arg	His	Arg
915								920				925			
His	Val	Pro	Pro	Ser	Phe	Arg	Val	Met	Val	Ser	Gly	Leu			
930								935				940			

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<210> SEQ ID NO 48
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Peptide

<400> SEQUENCE: 48
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Pro Leu Tyr Ser Ile Leu Ser Ala Leu Thr Ile Leu Gly Met Ile Met  
1 5 10 15  
Ala Ser Ala Phe Leu Phe Phe Asn Ile Lys Asn  
20 25

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<210> SEQ ID NO 49  
<211> LENGTH: 27  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence: Peptide

<400> SEQUENCE: 49

Leu Ile Ile Leu Gly Gly Met Leu Ser Tyr Ala Ser Ile Phe Leu Phe  
1 5 10 15

Gly Leu Asp Gly Ser Phe Val Ser Glu Lys Thr  
20 25

<210> SEQ ID NO 50  
<211> LENGTH: 25  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence: Peptide

<400> SEQUENCE: 50

Cys Thr Val Arg Thr Trp Ile Leu Thr Val Gly Tyr Thr Thr Ala Phe  
1 5 10 15

Gly Ala Met Phe Ala Lys Thr Trp Arg  
20 25

<210> SEQ ID NO 51  
<211> LENGTH: 22  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence: Peptide

<400> SEQUENCE: 51

Gln Lys Leu Leu Val Ile Val Gly Gly Met Leu Leu Ile Asp Leu Cys  
1 5 10 15

Ile Leu Ile Cys Trp Gln  
20

<210> SEQ ID NO 52  
<211> LENGTH: 24  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence: Peptide

<400> SEQUENCE: 52

Met Thr Ile Trp Leu Gly Ile Val Tyr Ala Tyr Lys Gly Leu Leu Met  
1 5 10 15

Leu Phe Gly Cys Phe Leu Ala Trp  
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<210> SEQ ID NO 53  
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<212> TYPE: PRT  
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<223> OTHER INFORMATION: Description of Artificial Sequence: Peptide

<400> SEQUENCE: 53

Ala Leu Asn Asp Ser Lys Tyr Ile Gly Met Ser Val Tyr Asn Val Gly  
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-continued

Ile Met Cys Ile Ile Gly Ala Ala Val  
20 25

<210> SEQ ID NO 54  
<211> LENGTH: 25  
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<400> SEQUENCE: 54

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Ile Met Cys Ile Ile Gly Ala Ala Val  
20 25

<210> SEQ ID NO 55  
<211> LENGTH: 844  
<212> TYPE: PRT  
<213> ORGANISM: Rattus Sp.

<400> SEQUENCE: 55

Met Gly Pro Gly Gly Pro Cys Thr Pro Val Gly Trp Pro Leu Pro Leu  
1 5 10 15

Leu Leu Val Met Ala Ala Gly Val Ala Pro Val Trp Ala Ser His Ser  
20 25 30

Pro His Leu Pro Arg Pro His Pro Arg Val Pro Pro His Pro Ser Ser  
35 40 45

Glu Arg Arg Ala Val Tyr Ile Gly Ala Leu Phe Pro Met Ser Gly Gly  
50 55 60

Trp Pro Gly Gly Gln Ala Cys Gln Pro Ala Val Glu Met Ala Leu Glu  
65 70 75 80

Asp Val Asn Ser Arg Arg Asp Ile Leu Pro Asp Tyr Glu Leu Lys Leu  
85 90 95

Ile His His Asp Ser Lys Cys Asp Pro Gly Gln Ala Thr Lys Tyr Leu  
100 105 110

Tyr Glu Leu Leu Tyr Asn Asp Pro Ile Lys Ile Ile Leu Met Pro Gly  
115 120 125

Cys Ser Ser Val Ser Thr Leu Val Ala Glu Ala Ala Arg Met Trp Asn  
130 135 140

Leu Ile Val Leu Ser Tyr Gly Ser Ser Ser Pro Ala Leu Ser Asn Arg  
145 150 155 160

Gln Arg Phe Pro Thr Phe Phe Arg Thr His Pro Ser Ala Thr Leu His  
165 170 175

Asn Pro Thr Arg Val Lys Leu Phe Glu Lys Trp Gly Trp Lys Lys Ile  
180 185 190

Ala Thr Ile Gln Gln Thr Thr Glu Val Phe Thr Ser Thr Leu Asp Asp  
195 200 205

Leu Glu Glu Arg Val Lys Glu Ala Gly Ile Glu Ile Thr Phe Arg Gln  
210 215 220

Ser Phe Phe Ser Asp Pro Ala Val Pro Val Lys Asn Leu Lys Arg Gln  
225 230 235 240

Asp Ala Arg Ile Ile Val Gly Leu Phe Tyr Glu Thr Glu Ala Arg Lys  
245 250 255

Val Phe Cys Glu Val Tyr Lys Glu Arg Leu Phe Gly Lys Lys Tyr Val

-continued

260					265					270					
Trp	Phe	Leu	Ile	Gly	Trp	Tyr	Ala	Asp	Asn	Trp	Phe	Lys	Thr	Tyr	Asp
275							280					285			
Pro	Ser	Ile	Asn	Cys	Thr	Val	Glu	Glu	Met	Thr	Glu	Ala	Val	Glu	Gly
290						295					300				
His	Ile	Thr	Thr	Glu	Ile	Val	Met	Leu	Asn	Pro	Ala	Asn	Thr	Arg	Ser
305				310						315					320
Ile	Ser	Asn	Met	Thr	Ser	Gln	Glu	Phe	Val	Glu	Lys	Leu	Thr	Lys	Arg
				325					330					335	
Leu	Lys	Arg	His	Pro	Glu	Glu	Thr	Gly	Gly	Phe	Gln	Glu	Ala	Pro	Leu
			340					345						350	
Ala	Tyr	Asp	Ala	Ile	Trp	Ala	Leu	Ala	Leu	Ala	Leu	Asn	Lys	Thr	Ser
		355					360						365		
Gly	Gly	Gly	Gly	Arg	Ser	Gly	Val	Arg	Leu	Glu	Asp	Phe	Asn	Tyr	Asn
		370				375					380				
Asn	Gln	Thr	Ile	Thr	Asp	Gln	Ile	Tyr	Arg	Ala	Met	Asn	Ser	Ser	Ser
385						390					395				400
Phe	Glu	Gly	Val	Ser	Gly	His	Val	Val	Phe	Asp	Ala	Ser	Gly	Ser	Arg
				405					410					415	
Met	Ala	Trp	Thr	Leu	Ile	Glu	Gln	Leu	Gln	Gly	Gly	Ser	Tyr	Lys	Lys
			420					425						430	
Ile	Gly	Tyr	Tyr	Asp	Ser	Thr	Lys	Asp	Asp	Leu	Ser	Trp	Ser	Lys	Thr
			435				440						445		
Asp	Lys	Trp	Ile	Gly	Gly	Ser	Pro	Pro	Ala	Asp	Gln	Thr	Leu	Val	Ile
		450				455							460		
Lys	Thr	Phe	Arg	Phe	Leu	Ser	Gln	Lys	Leu	Phe	Ile	Ser	Val	Ser	Val
465				470							475				480
Leu	Ser	Ser	Leu	Gly	Ile	Val	Leu	Ala	Val	Val	Cys	Leu	Ser	Phe	Asn
			485						490					495	
Ile	Tyr	Asn	Ser	His	Val	Arg	Tyr	Ile	Gln	Asn	Ser	Gln	Pro	Asn	Leu
		500						505					510		
Asn	Asn	Leu	Thr	Ala	Val	Gly	Cys	Ser	Leu	Ala	Leu	Ala	Ala	Val	Phe
		515					520						525		
Pro	Leu	Gly	Leu	Asp	Gly	Tyr	His	Ile	Gly	Arg	Ser	Gln	Phe	Pro	Phe
		530				535						540			
Val	Cys	Gln	Ala	Arg	Leu	Trp	Leu	Leu	Gly	Leu	Gly	Phe	Ser	Leu	Gly
545				550					555					560	
Tyr	Gly	Ser	Met	Phe	Thr	Lys	Ile	Trp	Trp	Val	His	Thr	Val	Phe	Thr
			565					570						575	
Lys	Lys	Glu	Glu	Lys	Lys	Glu	Trp	Arg	Lys	Thr	Leu	Glu	Pro	Trp	Lys
			580					585					590		
Leu	Tyr	Ala	Thr	Val	Gly	Leu	Leu	Val	Gly	Met	Asp	Val	Leu	Thr	Leu
		595					600						605		
Ala	Ile	Trp	Gln	Ile	Val	Asp	Pro	Leu	His	Arg	Thr	Ile	Glu	Thr	Phe
		610					615						620		
Ala	Lys	Glu	Glu	Pro	Lys	Glu	Asp	Ile	Asp	Val	Ser	Ile	Leu	Pro	Gln
625				630					635					640	
Leu	Glu	His	Cys	Ser	Ser	Lys	Lys	Met	Asn	Thr	Trp	Leu	Gly	Ile	Phe
			645					650						655	
Tyr	Gly	Tyr	Lys	Gly	Leu	Leu	Leu	Leu	Leu	Gly	Ile	Phe	Leu	Ala	Tyr
			660				665						670		

-continued

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Glu Thr Lys Ser Val Ser Thr	Glu Lys Ile Asn Asp His Arg Ala Val
675	680 685
Gly Met Ala Ile Tyr Asn Val Ala Val Leu Cys Leu Ile Thr Ala Pro	
690	695 700
Val Thr Met Ile Leu Ser Ser Gln Gln Asp Ala Ala Phe Ala Phe Ala	
705	710 715 720
Ser Leu Ala Ile Val Phe Ser Ser Tyr Ile Thr Leu Val Val Leu Phe	
725	730 735
Val Pro Lys Met Arg Arg Leu Ile Thr Arg Gly Glu Trp Gln Ser Glu	
740	745 750
Thr Gln Asp Thr Met Lys Thr Gly Ser Ser Thr Asn Asn Asn Glu Glu	
755	760 765
Glu Lys Ser Arg Leu Leu Glu Lys Glu Asn Arg Glu Leu Glu Lys Ile	
770	775 780
Ile Ala Glu Lys Glu Glu Arg Val Ser Glu Leu Arg His Gln Leu Gln	
785	790 795 800
Ser Arg Gln Gln Leu Arg Ser Arg Arg His Pro Pro Thr Pro Pro Asp	
805	810 815
Pro Ser Gly Glu Leu Pro Arg Gly Pro Ser Glu Pro Pro Asp Arg Leu	
820	825 830
Ser Cys Asp Gly Ser Arg Val His Leu Leu Tyr Lys	
835	840

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What is claimed is:

1. An isolated nucleic acid encoding a GABA<sub>B</sub>R2 polypeptide.
2. The nucleic acid of claim 1, wherein the nucleic acid is DNA.
3. The DNA of claim 2, wherein the DNA is cDNA.
4. The DNA of claim 2, wherein the DNA is genomic DNA.
5. The nucleic acid of claim 1, wherein the nucleic acid is RNA.
6. The nucleic acid of claim 1, wherein the nucleic acid encodes a mammalian GABA<sub>B</sub>R2 polypeptide.
7. The nucleic acid of claim 1, wherein the nucleic acid encodes a rat GABA<sub>B</sub>R2 polypeptide.
8. The nucleic acid of claim 1, wherein the nucleic acid encodes a human GABA<sub>B</sub>R2 polypeptide.
9. The nucleic acid of claim 6, wherein the nucleic acid encodes a polypeptide characterized by an amino acid sequence in the transmembrane regions which has an identity of 90% or higher to the amino acid sequence in the transmembrane regions of the human GABA<sub>B</sub>R2 polypeptide shown in FIGS. 5A-5D.
10. The nucleic acid of claim 6, wherein the nucleic acid encodes a mammalian GABA<sub>B</sub>R2 polypeptide which has substantially the same amino acid sequence as does the GABA<sub>B</sub>R2 polypeptide encoded by the plasmid BO-55 (ATCC Accession No. 209104).
11. The nucleic acid of claim 7, wherein the nucleic acid encodes a rat GABA<sub>B</sub>R2 polypeptide which has an amino acid sequence encoded by the plasmid BO-55 (ATCC Accession No. 209104).
12. The nucleic acid of claim 7, wherein the nucleic acid encodes a rat GABA<sub>B</sub>R2 polypeptide having substantially

the same amino acid sequence as the amino acid sequence shown in FIGS. 4A-4D (SEQ ID NO:4).

13. The nucleic acid of claim 7, wherein the rat GABA<sub>B</sub>R2 polypeptide has an amino acid sequence which comprises the amino acid sequence shown in FIGS. 4A-4D (SEQ ID NO:4).

14. The nucleic acid of claim 6, wherein the nucleic acid encodes a mammalian GABA<sub>B</sub>R2 polypeptide which has substantially the same amino acid sequence as does the GABA<sub>B</sub>R2 polypeptide encoded by the plasmid pEXJT3T7-hGABAB2 (ATCC Accession No. 203515).

15. The nucleic acid of claim 8, wherein the human GABA<sub>B</sub>R2 polypeptide comprises an amino acid sequence substantially the same as the amino acid sequence encoded by plasmid pEXJT3T7-hGABAB2 (ATCC Accession No. 203515).

16. The nucleic acid of claim 8, wherein the human GABA<sub>B</sub>R2 polypeptide comprises an amino acid sequence substantially the same as the amino acid sequence in FIGS. 23A-23D (SEQ ID NO:47).

17. The nucleic acid of claim 8, wherein the human GABA<sub>B</sub>R2 polypeptide has an amino acid sequence which comprises the sequence shown in FIGS. 23A-23D (SEQ ID NO:47).

18. A purified GABA<sub>B</sub>R2 protein.

19. A vector comprising the nucleic acid of claim 1.

20. A vector comprising the nucleic acid of claim 8.

21. A vector of claim 19 adapted for expression in a bacterial cell which comprises the regulatory elements necessary for expression of the nucleic acid in the bacterial cell operatively linked to the nucleic acid encoding a GABA<sub>B</sub>R2 polypeptide so as to permit expression thereof.

22. A vector of claim 19 adapted for expression in an amphibian cell which comprises the regulatory elements necessary for expression of the nucleic acid in the amphibian cell operatively linked to the nucleic acid encoding a GABA<sub>B</sub>R2 polypeptide so as to permit expression thereof.

23. A vector of claim 19 adapted for expression in a yeast cell which comprises the regulatory elements necessary for expression of the nucleic acid in the yeast cell operatively linked to the nucleic acid encoding a GABA<sub>B</sub>R2 polypeptide so as to permit expression thereof.

24. A vector of claim 19 adapted for expression in an insect cell which comprises the regulatory elements necessary for expression of the nucleic acid in the insect cell operatively linked to the nucleic acid encoding the GABA<sub>B</sub>R2 polypeptide so as to permit expression thereof.

25. A vector of claim 24 which is a baculovirus.

26. A vector of claim 19 adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the nucleic acid in the mammalian cell operatively linked to the nucleic acid encoding a GABA<sub>B</sub>R2 polypeptide so as to permit expression thereof.

27. A vector of claim 19 wherein the vector is a plasmid.

28. The plasmid of claim 27 designated BO-55 (ATCC Accession No. 209104).

29. The plasmid of claim 27 designated pEXJT3T7-hGABAB2 (ATCC Accession No. 203515).

30. A method of detecting a nucleic acid encoding a GABA<sub>B</sub>R2 polypeptide, which comprises contacting the nucleic acid with a probe comprising at least 15 nucleotides, which probe specifically hybridizes with the nucleic acid encoding the GABA<sub>B</sub>R2 polypeptide, wherein the probe has a unique sequence, which sequence is present within one of the two strands of the nucleic acid encoding the GABA<sub>B</sub>R2 polypeptide contained in plasmid BO-55, and detecting hybridization of the probe to the nucleic acid.

31. A method of detecting a nucleic acid encoding a GABA<sub>B</sub>R2 polypeptide, which comprises contacting the nucleic acid with a probe comprising at least 15 nucleotides, which probe specifically hybridizes with the nucleic acid encoding the GABA<sub>B</sub>R2 polypeptide, wherein the probe has a unique sequence, which sequence is present within (a) the nucleic acid sequence shown in FIGS. 22A-22D (SEQ ID NO:46) or (b) the reverse complement to the nucleic acid sequence shown in FIGS. 22A-22D (SEQ ID NO:46), and detecting hybridization of the probe to the nucleic acid.

32. A method of detecting a nucleic acid encoding a GABA<sub>B</sub>R2 polypeptide, which comprises contacting the nucleic acid with a probe comprising at least 15 nucleotides, which probe specifically hybridizes with the nucleic acid encoding the GABA<sub>B</sub>R2 polypeptide, wherein the probe has a unique sequence, which sequence is present within one of the two strands of the nucleic acid encoding the GABA<sub>B</sub>R2 polypeptide contained in plasmid pEXJT3T7-hGABAB2, and detecting hybridization of the probe to the nucleic acid.

33. A method of detecting a nucleic acid encoding a GABA<sub>B</sub>R2 polypeptide, which comprises contacting the nucleic acid with a probe comprising at least 15 nucleotides, which probe specifically hybridizes with the nucleic acid encoding the GABA<sub>B</sub>R2 polypeptide, wherein the probe has a unique sequence, which sequence is present within (a) the nucleic acid sequence shown in FIGS. 3A-3D (SEQ ID NO:3) or (b) the reverse complement to the nucleic acid sequence shown in FIGS. 3A-3D (SEQ ID NO:3), and detecting hybridization of the probe to the nucleic acid.

34. The method of any one of claims 30 to 33, wherein the nucleic acid is DNA.

35. The method of any one of claims 30 to 33, wherein the nucleic acid is RNA.

36. The method of any one of claims 30 to 33, wherein the probe comprises at least 15 nucleotides complementary to a unique segment of the sequence of the nucleic acid molecule encoding the GABA<sub>B</sub>R2 polypeptide.

37. A method of detecting a nucleic acid encoding a GABA<sub>B</sub>R2 polypeptide, which comprises contacting the nucleic acid with a probe comprising a nucleic acid of at least 15 nucleotides which is complementary to the antisense sequence of a unique segment of the sequence of the nucleic acid encoding the GABA<sub>B</sub>R2 polypeptide, and detecting hybridization of the probe to the nucleic acid.

38. A method of inhibiting translation of mRNA encoding a GABA<sub>B</sub>R2 polypeptide which comprises contacting such mRNA with an antisense oligonucleotide having a sequence capable of specifically hybridizing to the mRNA of claim 5, so as to prevent translation of the mRNA.

39. A method of inhibiting translation of mRNA encoding a GABA<sub>B</sub>R2 polypeptide which comprises contacting such mRNA with an antisense oligonucleotide having a sequence capable of specifically hybridizing to the genomic DNA of claim 4.

40. The method of claim 38 or 39, wherein the oligonucleotide comprises chemically modified nucleotides or nucleotide analogues.

41. An isolated antibody capable of binding to a GABA<sub>B</sub>R2 polypeptide encoded by the nucleic acid of claim 1.

42. The antibody of claim 41, wherein the GABA<sub>B</sub>R2 polypeptide is a human GABA<sub>B</sub>R2 polypeptide.

43. An antibody capable of competitively inhibiting the binding of the antibody of claim 41 to a GABA<sub>B</sub>R2 polypeptide.

44. An antibody of claim 41, wherein the antibody is a monoclonal antibody.

45. A monoclonal antibody of claim 44 directed to an epitope of a GABA<sub>B</sub>R2 polypeptide present on the surface of a GABA<sub>B</sub>R2 polypeptide expressing cell.

46. A method of claim 38 or 39, wherein the oligonucleotide is coupled to a substance which inactivates mRNA.

47. A method of claim 46, wherein the substance which inactivates mRNA is a ribozyme.

48. A pharmaceutical composition which comprises an amount of the antibody of claim 41 effective to block binding of a ligand to the GABA<sub>B</sub>R2 polypeptide and a pharmaceutically acceptable carrier.

49. A transgenic, nonhuman mammal expressing DNA encoding a GABA<sub>B</sub>R2 polypeptide of claim 1.

50. A transgenic, nonhuman mammal comprising a homologous recombination knockout of the native GABA<sub>B</sub>R2 polypeptide.

51. A transgenic, nonhuman mammal whose genome comprises antisense DNA complementary to DNA encoding a GABA<sub>B</sub>R2 polypeptide of claim 1 so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding such GABA<sub>B</sub>R2 polypeptide and which hybridizes to such mRNA encoding such GABA<sub>B</sub>R2 polypeptide, thereby reducing its translation.

52. The transgenic, nonhuman mammal of claim 49 or 50, wherein the DNA encoding the GABA<sub>B</sub>R2 polypeptide additionally comprises an inducible promoter.

53. The transgenic, nonhuman mammal of claim 49 or 50, wherein the DNA encoding the GABA<sub>B</sub>R2 polypeptide additionally comprises tissue specific regulatory elements.

54. A transgenic, nonhuman mammal of any one of claims 49, 50 or 51, wherein the transgenic, nonhuman mammal is a mouse.

55. A method of detecting the presence of a GABA<sub>B</sub>R2 polypeptide on the surface of a cell which comprises contacting the cell with the antibody of claim 41 under conditions permitting binding of the antibody to the polypeptide, detecting the presence of the antibody bound to the cell, and thereby detecting the presence of a GABA<sub>B</sub>R2 polypeptide on the surface of the cell.

56. A method of preparing the purified GABA<sub>B</sub>R2 polypeptide of claim 18 which comprises:

- a. inducing cells to express a GABA<sub>B</sub>R2 polypeptide;
- b. recovering the polypeptide so expressed from the induced cells; and
- c. purifying the polypeptide so recovered.

57. A method of preparing the purified GABA<sub>B</sub>R2 polypeptide of claim 18 which comprises:

- a. inserting a nucleic acid encoding the GABA<sub>B</sub>R2 polypeptide into a suitable vector;
- b. introducing the resulting vector in a suitable host cell;
- c. placing the resulting cell in suitable condition permitting the production of the GABA<sub>B</sub>R2 polypeptide;
- d. recovering the polypeptide produced by the resulting cell; and
- e. isolating or purifying the polypeptide so recovered.

58. A GABA<sub>B</sub>R1/R2 receptor comprising two polypeptides, one of which is a GABA<sub>B</sub>R2 polypeptide and another of which is a GABA<sub>B</sub>R1 polypeptide.

59. A method of forming a GABA<sub>B</sub>R1/R2 receptor which comprises inducing cells to express both a GABA<sub>B</sub>R1 polypeptide and a GABA<sub>B</sub>R2 polypeptide.

60. An antibody capable of binding to a GABA<sub>B</sub>R1/R2 receptor, wherein the GABA<sub>B</sub>R2 polypeptide is encoded by the nucleic acid of claim 1.

61. The antibody of claim 60, wherein the GABA<sub>B</sub>R2 polypeptide is a human GABA<sub>B</sub>R2 polypeptide.

62. An antibody capable of competitively inhibiting the binding of the antibody of claim 60 to a GABA<sub>B</sub>R1/R2 receptor.

63. An antibody of claim 60, wherein the antibody is a monoclonal antibody.

64. A monoclonal antibody of claim 63 directed to an epitope of a GABA<sub>B</sub>R1/R2 receptor present on the surface of a GABA<sub>B</sub>R1/R2 polypeptide expressing cell.

65. A pharmaceutical composition which comprises an amount of the antibody of claim 60 effective to block binding of a ligand to the GABA<sub>B</sub>R1/R2 receptor and a pharmaceutically acceptable carrier.

66. A transgenic, nonhuman mammal expressing a GABA<sub>B</sub>R1/R2 receptor, which is not naturally expressed by the mammal.

67. A transgenic, nonhuman mammal comprising a homologous recombination knockout of the native GABA<sub>B</sub>R1/R2 receptor.

68. A transgenic, nonhuman mammal of claim 66 or 67, wherein the transgenic nonhuman mammal is a mouse.

69. A method of detecting the presence of a GABA<sub>B</sub>R1/R2 receptor on the surface of a cell which comprises contacting the cell with the antibody of claim 60 under conditions permitting binding of the antibody to the receptor, detecting the presence of the antibody bound to the cell, and thereby detecting the presence of a GABA<sub>B</sub>R1/R2 receptor on the surface of the cell.

70. A method of determining the physiological effects of varying levels of activity of GABA<sub>B</sub>R1/R2 receptors which comprises producing a transgenic nonhuman mammal of claim 66 whose levels of GABA<sub>B</sub>R1/R2 receptor activity vary due to the presence of an inducible promoter which regulates GABA<sub>B</sub>R1/R2 receptor expression.

71. A method of determining the physiological effects of varying levels of activity of GABA<sub>B</sub>R1/R2 receptors which comprises producing a panel of transgenic nonhuman mammals of claim 66, each expressing a different amount of GABA<sub>B</sub>R1/R2 receptor.

72. A method for identifying an antagonist capable of alleviating an abnormality, by decreasing the activity of a GABA<sub>B</sub>R1/R2 receptor comprising administering a compound to the transgenic nonhuman mammal of claim 66 or 68, and determining whether the compound alleviates the physical and behavioral abnormalities displayed by the transgenic, nonhuman mammal, the alleviation of the abnormality identifying the compound as the antagonist.

73. An antagonist identified by the method of claim 72.

74. A pharmaceutical composition comprising an antagonist of claim 73 and a pharmaceutically acceptable carrier.

75. A method of treating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of a GABA<sub>B</sub>R1/R2 receptor which comprises administering to a subject an effective amount of the pharmaceutical composition of claim 74, thereby treating the abnormality.

76. A method for identifying an agonist capable of alleviating an abnormality, by increasing the activity of a GABA<sub>B</sub>R1/R2 receptor comprising administering a compound to the transgenic nonhuman mammal of claim 66 or 68, and determining whether the compound alleviates the physical and behavioral abnormalities displayed by the transgenic, nonhuman mammal, the alleviation of the abnormality identifying the compound as the agonist.

77. An agonist identified by the method of claim 76.

78. A pharmaceutical composition comprising an agonist of claim 76 and a pharmaceutically acceptable carrier.

79. A method for treating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a GABA<sub>B</sub>R1/R2 receptor which comprises administering to a subject an effective amount of the pharmaceutical composition of claim 78, thereby treating the abnormality.

80. A cell which expresses on its surface a mammalian GABA<sub>B</sub>R1/R2 receptor that is not naturally expressed on the surface of such cell.

81. A cell of claim 80, wherein the mammalian GABA<sub>B</sub>R1/R2 receptor comprises two polypeptides, one of which is a GABA<sub>B</sub>R2 polypeptide and another of which is a GABA<sub>B</sub>R1 polypeptide.

82. A process for identifying a chemical compound which specifically binds to a GABA<sub>B</sub>R1/R2 receptor which comprises contacting cells containing nucleic acid encoding and expressing on their cell surface the GABA<sub>B</sub>R1/R2 receptor, wherein such cells do not normally express the GABA<sub>B</sub>R1/R2 receptor.

R2 receptor, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the GABA<sub>B</sub>R1/R2 receptor.

83. A process for identifying a chemical compound which specifically binds to a GABA<sub>B</sub>R1/R2 receptor which comprises contacting a membrane fraction from a cell extract of cells containing nucleic acid encoding and expressing on their cell surface the GABA<sub>B</sub>R1/R2 receptor, wherein such cells do not normally express the GABA<sub>B</sub>R1/R2 receptor, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the GABA<sub>B</sub>R1/R2 receptor.

84. The process of claim 82 or 83, wherein the GABA<sub>B</sub>R1/R2 receptor is a mammalian GABA<sub>B</sub>R1/R2 receptor.

85. The process of claim 82 or 83, wherein the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has substantially the same amino acid sequence as that encoded by the plasmid BO-55 (ATCC Accession No. 209104).

86. The process of claim 82 or 83, wherein the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has substantially the same sequence as the amino acid sequence shown in FIGS. 23A-23D (SEQ ID NO:47).

87. The process of claim 82 or 83, wherein the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has the amino acid sequence shown in FIGS. 23A-23D (SEQ ID NO:47).

88. The process of claims 82 or 83, wherein the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has substantially the same amino acid sequence as that encoded by the plasmid pEXJT3T7-hGABAB2 (ATCC Accession No. 203515).

89. The process of claim 82 or 83, wherein the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has substantially the same amino acid sequence as the sequence shown in FIGS. 23A-23D (SEQ ID NO:47).

90. The process of claim 82 or 83, wherein the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has the sequence shown in FIGS. 23A-23D (SEQ ID NO:47).

91. The process of claim 89, wherein the compound is not previously known to bind to a GABA<sub>B</sub>R1/R2 receptor.

92. A compound identified by the process of claim 91.

93. A process of claim 89, wherein the cell is an insect cell.

94. A process of claim 89, wherein the cell is a mammalian cell.

95. A process of claim 94, wherein the cell is nonneuronal in origin.

96. A process of claim 95, wherein the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell a mouse Y1 cell or LM(tk-) cell.

97. A process of claim 94, wherein the compound is not previously known to bind to a GABA<sub>B</sub>R1/R2 receptor.

98. A compound identified by the process of claim 97.

99. A process involving competitive binding for identifying a chemical compound which specifically binds to a GABA<sub>B</sub>R1/R2 receptor which comprises separately contacting cells expressing on their cell surface the GABA<sub>B</sub>R1/R2 receptor, wherein such cells do not normally express the GABA<sub>B</sub>R1/R2 receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds,

and detecting specific binding of the chemical compound to the GABA<sub>B</sub>R1/R2 receptor, a decrease in the binding of the second chemical compound to the GABA<sub>B</sub>R1/R2 receptor in the presence of the chemical compound indicating that the chemical compound binds to the GABA<sub>B</sub>R1/R2 receptor.

100. A process involving competitive binding for identifying a chemical compound which specifically binds to a human GABA<sub>B</sub>R1/R2 receptor which comprises separately contacting a membrane fraction from a cell extract of cells expressing on their cell surface the GABA<sub>B</sub>R1/R2 receptor, wherein such cells do not normally express the GABA<sub>B</sub>R1/R2 receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the chemical compound to the GABA<sub>B</sub>R1/R2 receptor, a decrease in the binding of the second chemical compound to the GABA<sub>B</sub>R1/R2 receptor in the presence of the chemical compound indicating that the chemical compound binds to the GABA<sub>B</sub>R1/R2 receptor.

101. A process of claim 99 or 100, wherein the GABA<sub>B</sub>R1/R2 receptor is a mammalian GABA<sub>B</sub>R1/R2 receptor.

102. The process of claim 101, wherein the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has substantially the same amino acid sequence as that encoded by plasmid BO-55 (ATCC Accession No. 209104).

103. The process of claim 99 or 100, wherein the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has substantially the same amino acid sequence as that shown in FIGS. 23A-23D (SEQ ID NO:47).

104. The process of claim 99 or 100, wherein the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has the amino acid sequence shown in FIGS. 23A-23D (Seq. ID NO:47).

105. The process of claim 99 or 100, wherein the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has substantially the same amino acid sequence as that encoded by plasmid pEXJT3T7-hGABAB2 (ATCC Accession No.203515).

106. The process of claim 99 or 100, wherein the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has substantially the same amino acid sequence as the sequence shown in FIGS. 23A-23D (SEQ ID NO:47).

107. The process of claim 99 or 100, wherein the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has the sequence shown in FIGS. 23A-23D (SEQ ID NO:47).

108. The process of claim 107, wherein the cell is an insect cell.

109. The process of claim 107, wherein the cell is a mammalian cell.

110. The process of claim 109, wherein the cell is nonneuronal in origin.

111. The process of claim 110, wherein the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell a mouse Y1 cell or LM(tk-) cell.

112. The process of claim 109, wherein the compound is not previously known to bind to a GABA<sub>B</sub>R1/R2 receptor.

113. A compound identified by the process of claim 112.

114. A method of screening a plurality of chemical compounds not known to bind to a GABA<sub>B</sub>R1/R2 receptor to identify a compound which specifically binds to the GABA<sub>B</sub>R1/R2 receptor, which comprises



- (a) contacting cells containing nucleic acid encoding and expressing on their cell surface the GABA<sub>B</sub>R1/R2 receptor, wherein such cells do not normally express the GABA<sub>B</sub>R1/R2 receptor, with a compound known to bind specifically to the GABA<sub>B</sub>R1/R2 receptor;
- (b) contacting the same cells as in step (a) with the plurality of compounds not known to bind specifically to the GABA<sub>B</sub>R1/R2 receptor, under conditions permitting binding of compounds known to bind the GABA<sub>B</sub>R1/R2 receptor;
- (c) determining whether the binding of the compound known to bind specifically to the GABA<sub>B</sub>R1/R2 receptor is reduced in the presence of the plurality of the compounds, relative to the binding of the compound in the absence of the plurality of compounds, and if the binding is reduced;
- (d) separately determining the extent of binding to the GABA<sub>B</sub>R1/R2 receptor of each compound included in the plurality of compounds, so as to thereby identify the compound or compounds present in such plurality of compounds which specifically binds to the GABA<sub>B</sub>R1/R2 receptor.

115. A method of screening a plurality of chemical compounds not known to bind to a GABA<sub>B</sub>R1/R2 receptor to identify a compound which specifically binds to the GABA<sub>B</sub>R1/R2 receptor, which comprises

- (a) contacting a membrane fraction extract from cells containing nucleic acid encoding and expressing on their cell surface the GABA<sub>B</sub>R1/R2 receptor, wherein such cells do not normally express the GABA<sub>B</sub>R1/R2 receptor, with a compound known to bind specifically to the GABA<sub>B</sub>R1/R2 receptor;
- (b) contacting the same membrane fraction as in step (a) with the plurality of compounds not known to bind specifically to the GABA<sub>B</sub>R1/R2 receptor, under conditions permitting binding of compounds known to bind the GABA<sub>B</sub>R1/R2 receptor;
- (c) determining whether the binding of the compound known to bind specifically to the GABA<sub>B</sub>R1/R2 receptor is reduced in the presence of the plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds, and if the binding is reduced;
- (d) separately determining the extent of binding to the GABA<sub>B</sub>R1/R2 receptor of each compound included in the plurality of compounds, so as to thereby identify the compound or compounds present in such plurality of compounds which specifically binds to the GABA<sub>B</sub>R1/R2 receptor.

116. A method of claim 114 or 115, wherein the GABA<sub>B</sub>R1/R2 receptor is a mammalian GABA<sub>B</sub>R1/R2 receptor.

117. A method of either of claim 114 or 115, wherein the cell is a mammalian cell.

118. A method of claim 117, wherein the mammalian cell is non-neuronal in origin.

119. The method of claim 118, wherein the non-neuronal cell is a COS-7 cell, a 293 human embryonic kidney cell, a LM(tk-) cell, a CHO cell, a mouse Y1 cell or an NIH-3T3 cell.

120. A process for determining whether a chemical compound is a GABA<sub>B</sub>R1/R2 receptor agonist which comprises contacting cells with the compound under conditions permitting the activation of the GABA<sub>B</sub>R1/R2 receptor, and detecting an increase in GABA<sub>B</sub>R1/R2 receptor activity, so as to thereby determine whether the compound is a GABA<sub>B</sub>R1/R2 receptor agonist.

121. A process for determining whether a chemical compound is a GABA<sub>B</sub>R1/R2 receptor antagonist which comprises contacting cells containing nucleic acid encoding and expressing on their cell surface the GABA<sub>B</sub>R1/R2 receptor, wherein such cells do not normally express the GABA<sub>B</sub>R1/R2 receptor, with the compound in the presence of a known GABA<sub>B</sub>R1/R2 receptor agonist, under conditions permitting the activation of the GABA<sub>B</sub>R1/R2 receptor, and detecting a decrease in GABA<sub>B</sub>R1/R2 receptor activity, so as to thereby determine whether the compound is a GABA<sub>B</sub>R1/R2 receptor antagonist.

122. A process of claim 120 or 121, wherein the cells additionally express nucleic acid encoding GIRK1 and GIRK4.

123. A process of any one of claims 120, 121, or 122, wherein the GABA<sub>B</sub>R2 receptor is a mammalian GABA<sub>B</sub>R2 receptor.

124. A pharmaceutical composition which comprises an amount of a GABA<sub>B</sub>R1/R2 receptor agonist determined to be an agonist by the process of claim 120 effective to increase activity of a GABA<sub>B</sub>R1/R2 receptor and a pharmaceutically acceptable carrier.

125. A pharmaceutical composition of claim 124, wherein the GABA<sub>B</sub>R1/R2 receptor agonist was not previously known.

126. A pharmaceutical composition which comprises an amount of a GABA<sub>B</sub>R1/R2 receptor antagonist determined to be an antagonist the process of claim 121 effective to reduce activity of a GABA<sub>B</sub>R1/R2 receptor and a pharmaceutically acceptable carrier.

127. A pharmaceutical composition of claim 126, wherein the GABA<sub>B</sub>R1/R2 receptor antagonist was not previously known.

128. A process for determining whether a chemical compound activates a GABA<sub>B</sub>R1/R2 receptor, which comprises contacting cells producing a second messenger response and expressing on their cell surface the GABA<sub>B</sub>R1/R2 receptor, wherein such cells do not normally express the GABA<sub>B</sub>R1/R2 receptor, with the chemical compound under conditions suitable for activation of the GABA<sub>B</sub>R1/R2 receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change in the second messenger response in the presence of the chemical compound indicating that the compound activates the GABA<sub>B</sub>R1/R2 receptor.

129. The process of claim 128, wherein the second messenger response comprises potassium channel activation and the change in second messenger is an increase in the level of potassium current.

130. A process for determining whether a chemical compound inhibits activation of a GABA<sub>B</sub>R1/R2 receptor, which comprises separately contacting cells producing a second messenger response and expressing on their cell surface the GABA<sub>B</sub>R1/R2 receptor, wherein such cells do not normally express the GABA<sub>B</sub>R1/R2 receptor, with both the chemical compound and a second chemical compound known to activate the GABA<sub>B</sub>R1/R2 receptor, and with only

the second chemical compound, under conditions suitable for activation of the GABA<sub>B</sub>R1/R2 receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change in the second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the GABA<sub>B</sub>R1/R2 receptor.

131. The process of claim 130, wherein the second messenger response comprises potassium channel activation and the change in second messenger response is a smaller increase in the level of inward potassium current in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

132. A process of any one of claims 128, 129, 130 or 131, wherein the GABA<sub>B</sub>R1/R2 receptor is a mammalian GABA<sub>B</sub>R1/R2 receptor.

133. The process of claim 132, wherein the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has substantially the same amino acid sequence as that encoded by the plasmid BO-55 (ATCC Accession No. 209104).

134. The process of claim 132, wherein the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has substantially the same amino acid sequence as that shown in FIGS. 4A-4D (SEQ ID NO:4).

135. The process of claim 132, wherein the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has substantially the same amino acid sequence as that shown in FIGS. 23A-23D (SEQ ID NO:47).

136. The process of claim 132, wherein the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has the sequence, shown in FIGS. 23A-23D (SEQ ID NO:47).

137. The process of claim 132, wherein the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has substantially the same amino acid sequence as that encoded by the plasmid pEXJT3T7-hGABAB2 (ATCC Accession No. 203515).

138. The process of any one of claims 128-131, wherein the cell is an insect cell.

139. The process of any one of claims 128-131, wherein the cell is a mammalian cell.

140. The process of claim 139, wherein the mammalian cell is nonneuronal in origin.

141. The process of claim 140, wherein the nonneuronal cell is a COS-7 cell, CHO cell, 293 human embryonic kidney cell, NIH-3T3 cell or LM(tk-) cell.

142. The process of claim 139, wherein the compound was not previously known to activate or inhibit a GABA<sub>B</sub>R1/R2 receptor.

143. A compound determined by the process of claim 142.

144. A pharmaceutical composition which comprises an amount of a GABA<sub>B</sub>R1/R2 receptor agonist determined by the process of claim 128 or 129 effective to increase activity of a GABA<sub>B</sub>R1/R2 receptor and a pharmaceutically acceptable carrier.

145. A pharmaceutical composition of claim 144, wherein the GABA<sub>B</sub>R1/R2 receptor agonist was not previously known.

146. A pharmaceutical composition which comprises an amount of a GABA<sub>B</sub>R1/R2 receptor antagonist determined

by the process of claim 130 or 131 effective to reduce activity of a GABA<sub>B</sub>R1/R2 receptor and a pharmaceutically acceptable carrier.

147. A pharmaceutical composition of claim 146, wherein the GABA<sub>B</sub>R1/R2 receptor antagonist was not previously known.

148. A method of screening a plurality of chemical compounds not known to activate a GABA<sub>B</sub>R1/R2 receptor to identify a compound which activates the GABA<sub>B</sub>R1/R2 receptor which comprises:

- (a) contacting cells containing nucleic acid encoding and expressing on their cell surface the GABA<sub>B</sub>R1/R2 receptor, wherein such cells do not normally express the GABA<sub>B</sub>R1/R2 receptor, with the plurality of compounds not known to activate the GABA<sub>B</sub>R1/R2 receptor, under conditions permitting activation of the GABA<sub>B</sub>R1/R2 receptor;
- (b) determining whether the activity of the GABA<sub>B</sub>R1/R2 receptor is increased in the presence of the compounds, and if it is increased;
- (c) separately determining whether the activation of the GABA<sub>B</sub>R1/R2 receptor is increased by each compound included in the plurality of compounds, so as to thereby identify the compound or compounds present in such plurality of compounds which activates the GABA<sub>B</sub>R1/R2 receptor.

149. The process of claim 148, wherein the cells express nucleic acid encoding GIRK1 and GIRK4.

150. A method of claim 148 or 149, wherein the GABA<sub>B</sub>R1/R2 receptor is a mammalian GABA<sub>B</sub>R1/R2 receptor.

151. A method of screening a plurality of chemical compounds not known to inhibit the activation of a GABA<sub>B</sub>R1/R2 receptor to identify a compound which inhibits the activation of the GABA<sub>B</sub>R1/R2 receptor, which comprises:

- (a) contacting cells containing nucleic acid encoding and expressing on their cell surface the GABA<sub>B</sub>R1/R2 receptor, wherein such cells do not normally express the GABA<sub>B</sub>R1/R2 receptor, with the plurality of compounds in the presence of a known GABA<sub>B</sub>R1/R2 receptor agonist, under conditions permitting activation of the GABA<sub>B</sub>R1/R2 receptor;
- (b) determining whether the activation of the GABA<sub>B</sub>R1/R2 receptor is reduced in the presence of the plurality of compounds, relative to the activation of the GABA<sub>B</sub>R1/R2 receptor in the absence of the plurality of compounds, and if it is reduced;
- (c) separately determining the inhibition of activation of the GABA<sub>B</sub>R1/R2 receptor for each compound included in the plurality of compounds, so as to thereby identify the compound or compounds present in such a plurality of compounds which inhibits the activation of the GABA<sub>B</sub>R1/R2 receptor.

152. The process of claim 151, wherein the cells express nucleic acid encoding GIRK1 and GIRK4.

153. A method of claim 151 or 152, wherein the GABA<sub>B</sub>R1/R2 receptor is a mammalian GABA<sub>B</sub>R1/R2 receptor.

154. A method of any one of claims 148, 149, 151, or 152, wherein the cell is a mammalian cell.

155. A method of claim 154, wherein the mammalian cell is non-neuronal in origin.

156. The method of claim 155, wherein the non-neuronal cell is a COS-7 cell, a 293 human embryonic kidney cell, a LM(tk-) cell or an NIH-3T3 cell.

157. A pharmaceutical composition comprising a compound identified by the method of claim 148 or 149, effective to increase GABA<sub>B</sub>R1/R2 receptor activity and a pharmaceutically acceptable carrier.

158. A pharmaceutical composition comprising a compound identified by the method of claim 151 or 152, effective to decrease GABA<sub>B</sub>R1/R2 receptor activity and a pharmaceutically acceptable carrier.

159. A process for determining whether a chemical compound is a GABA<sub>B</sub>R1/R2 receptor agonist, which comprises preparing a membrane fraction from cells which comprise nucleic acid encoding and expressing on their cell surface the GABA<sub>B</sub>R1/R2 receptor, wherein such cells do not normally express the GABA<sub>B</sub>R1/R2 receptor, separately contacting the membrane fraction with both the chemical compound and GTPYS, and with only GTPYS, under conditions permitting the activation of the GABA<sub>B</sub>R1/R2 receptor, and detecting GTPYS binding to the membrane fraction, an increase in GTPYS binding in the presence of the compound indicating that the chemical compound activates the GABA<sub>B</sub>R1/R2 receptor.

160. A process for determining whether a chemical compound is a GABA<sub>B</sub>R1/R2 receptor antagonist, which comprises preparing a membrane fraction from cells which comprise nucleic acid encoding and expressing on their cell surface the GABA<sub>B</sub>R1/R2 receptor, wherein such cells do not normally express the GABA<sub>B</sub>R1/R2 receptor, separately contacting the membrane fraction with the chemical compound, GTPYS and a second chemical compound known to activate the GABA<sub>B</sub>R1/R2 receptor, with GTPYS and only the second compound, and with GTPYS alone, under conditions permitting the activation of the GABA<sub>B</sub>R1/R2 receptor, detecting GTPYS binding to each membrane fraction, and comparing the increase in GTPYS binding in the presence of the compound and the second compound relative to the binding of GTPYS alone, to the increase in GTPYS binding in the presence of the second chemical compound known to activate the GABA<sub>B</sub>R1/R2 receptor relative to the binding of GTPYS alone, a smaller increase in GTPYS binding in the presence of the compound and the second compound indicating that the compound is a GABA<sub>B</sub>R1/R2 receptor antagonist.

161. A process of claim 159 or 160, wherein the GABA<sub>B</sub>R2 receptor is a mammalian GABA<sub>B</sub>R2 receptor.

162. The process of claim 161, wherein the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has substantially the same amino acid sequence as that encoded by the plasmid BO-55 (ATCC Accession No. 209104).

163. The process of claim 162, wherein the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has substantially the same amino acid sequence as that shown in FIGS. 4A-4D (SEQ ID NO:4).

164. The process of claim 161, wherein the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has substantially the same amino acid sequence as that encoded by the plasmid pEXJT3T7-hGABAB2 (ATCC Accession No. 203515).

165. The process of claim 161, wherein the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has

substantially the same amino acid sequence as that shown in FIGS. 23A-23D (SEQ ID NO:47).

166. The process of claim 161, wherein the GABA<sub>B</sub>R1/R2 receptor comprises a GABA<sub>B</sub>R2 polypeptide which has the sequence shown in FIGS. 23A-23D (SEQ ID NO:47).

167. The process of claim 159 or 160, wherein the cell is an insect cell.

168. The process of claim 159 or 160, wherein the cell is a mammalian cell.

169. The process of claim 168, wherein the mammalian cell is nonneuronal in origin.

170. The process of claim 169, wherein the nonneuronal cell is a COS-7 cell, CHO cell, 293 human embryonic kidney cell, NIH-3T3 cell or LM(tk-) cell.

171. The process of claim 170, wherein the compound was not previously known to be an agonist or antagonist of a GABA<sub>B</sub>R1/R2 receptor.

172. A compound determined to be an agonist or antagonist of a GABA<sub>B</sub>R1/R2 receptor by the process of claim 171.

173. A method of treating spasticity in a subject which comprises administering to the subject an amount of a compound which is an agonist of a GABA<sub>B</sub>R1/R2 receptor effective to treat spasticity in the subject.

174. A method of treating asthma in a subject which comprises administering to the subject an amount of a compound which is a GABA<sub>B</sub>R1/R2 receptor agonist effective to treat asthma in the subject.

175. A method of treating incontinence in a subject which comprises administering to the subject an amount of a compound which is a GABA<sub>B</sub>R1/R2 receptor agonist effective to treat incontinence in the subject.

176. A method of decreasing nociception in a subject which comprises administering to the subject an amount of a compound which is a GABA<sub>B</sub>R1/R2 receptor agonist effective to decrease nociception in the subject.

177. A use of a GABA<sub>B</sub>R2 agonist as an antitussive agent which comprises administering to the subject an amount of a compound which is a GABA<sub>B</sub>R1/R2 receptor agonist effective as an antitussive agent in the subject.

178. A method of treating drug addiction in a subject which comprises administering to the subject an amount of a compound which is a GABA<sub>B</sub>R1/R2 receptor agonist effective to treat drug addiction in the subject.

179. A method of treating Alzheimer's disease in a subject which comprises administering to the subject an amount of a compound which is a GABA<sub>B</sub>R1/R2 receptor antagonist effective to treat Alzheimer's disease in the subject.

182. A process for making a composition of matter which specifically binds to a GABA<sub>B</sub>R1/R2 receptor which comprises identifying a chemical compound using the process of any of claims, 82, 83, 99, 100, 114 or 115 and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof.

183. A process for making a composition of matter which specifically binds to a GABA<sub>B</sub>R1/R2 receptor which comprises identifying a chemical compound using the process of any of claims 120, 128, or 148 and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof.

184. A process for making a composition of matter which specifically binds to a GABA<sub>B</sub>R1/R2 receptor which comprises identifying a chemical compound using the process of

any of claims 121, 130, or 151 and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof.

185. The process of any of claims 182, 183, or 184, wherein the GABA<sub>B</sub>R1/R2 receptor is a human GABA<sub>B</sub>R1/R2 receptor.

186. A process for preparing a pharmaceutical composition which comprises admixing a pharmaceutically acceptable carrier and a pharmaceutically acceptable amount of a chemical compound identified by the process of any of claims 82, 83, 99, 100, 114 or 115 or a novel structural and functional analog or homolog thereof.

187. A process for preparing a pharmaceutical composition which comprises admixing a pharmaceutically acceptable carrier and a pharmaceutically acceptable amount of a

chemical compound identified by the process of any of claims 120, 128, or 148 or a novel structural and functional analog or homolog thereof.

188. A process for preparing a pharmaceutical composition which comprises admixing a pharmaceutically acceptable carrier and a pharmaceutically acceptable amount of a chemical compound identified by the process of any of claims 121, 130, or 151 or a novel structural and functional analog or homolog thereof.

189. The process of any of claims 186, 187, or 188, wherein the GABA<sub>B</sub>R1/R2 receptor is a human GABA<sub>B</sub>R1/R2 receptor.

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